ANTITUMOR AGENTS 57.[1] THE ISOLATION AND STRUCTURAL ELUCIDATION OF MICROHELENIN-E, A NEW ANTILEUKEMIC NOR-PSEUDOGUAIANOLIDE, AND MICROHELENIN-F FROM *HELENIUM MICROCEPHALUM*

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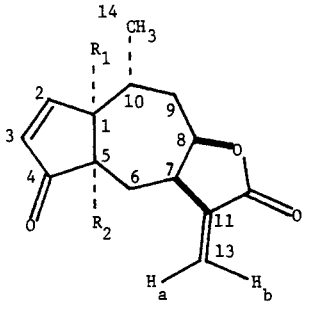
ABSTRACT.—Two new nor-pseudoguaianolides, microhelenin-E (**1**) and -F (**2**), were isolated from Texas *Helenium microcephalum* and their structures elucidated on the basis of physicochemical data and spectral evidence. Microhelenin-E demonstrated significant *in vitro* and *in vivo* cytotoxic and antileukemic activities against KB tissue cell culture (ED_{50}=1.38 μg/ml) and P-388 lymphocytic leukemia growth in BDF_1 male mice (T/C=166% at 8 mg/kg/day), respectively.

Previous investigation of the antitumor principles of Texas *Helenium microcephalum* has led to the isolation of new sesquiterpene lactones microhelenins -A, -B, -C, -D (i.e. mexicanin-E), microlenin, microlenin acetate, isohelenalin and isohelenol (1–6) in addition to helenalin (7) from this laboratory. In this paper, further isolation and structural elucidation of two new nor-pseudoguaianolides microhelenin-E (**1**) and -F (**2**) are reported.



1 R_1 = OH, R_2 = H (microhelenin-E)

2 R_1 = H, R_2 = OH (microhelenin-F)

3 R_1 = R_2 = H (mexicanin-E)

Microhelenins-E (**1**) and -F (**2**) were isolated by an initial repeated silica gel and Sephadex LH-20 column chromatographies of a chloroform extract of the whole plant of *H. microcephalum*, followed by a subsequent purification with high performance liquid chromatography in the recycle mode.

Microhelenin-E (**1**), mp 135–136.5°, has molecular formula $C_{14}H_{16}O_4$ as determined by exact mass molecular ion peak in the mass spectrum. The ir spectrum of **1** showed the presence of a cyclopentenone ring (1710 and 1590 cm^{-1}), an α,β-unsaturated-γ-lactone moiety (1760 and 1660 cm^{-1}) and a tertiary hydroxyl group (3450 cm^{-1}) which resisted acetylation with acetic anhydride in pyridine at room temperature. This was substantiated by the presence in the nmr spectrum (table 1) of a pair of low field doublets at δ 6.18 (1H, J=5.0 Hz, H-3) and 7.60 (1H, J=5.0 Hz, H-2) (cyclopentenone), and another pair of low field doublets at δ5.66 (1H, J=1.5 Hz, H-13_a) and 6.21 (1H, J=1.5 Hz, H-13_b) [typical α-methylene

[1]For part 56 see Y. F. Liou, I. H. Hall and K. H. Lee, *J. Pharm. Sci.*, submitted.

grouping of the γ-lactone bearing a proton at the β-position (H–7)]. A three-proton doublet at δ 1.16 (3H, $J=5$ Hz) was ascribed to the secondary methyl group at C–10. The one-proton signals at δ 4.86 (ddd, $J=3.0$, 4.0 and 4.0 Hz) and 3.19 (m) were assigned to the hydrogens at C–8 and C–7, respectively, as the chemical shifts and signal pattern of these protons were comparable to those of the corresponding known mexicanin-E (**3**), isolated previously from this same plant (2).

The identical sign of Cotton effects (CE)[2] in circular dichoism of **1** compared to that of mexicanin-E (**3**) whose absolute configuration had been established (2), indicated that **1** possessed the same *cis*-fused cyclopentenone and *cis*-fused α-methylene-γ-lactone ring systems as **3** (8, 9). In addition, Samek's rule (10) ($J_{7,13}$ *trans*-lactone $\geq 3 \geq J_{7,13}$ *cis*-lactone) also indicated that **1** contains a *cis*-

TABLE 1. ^{1}H-nmr spectra of microhelenin-E (**1**), microhelenin-F (**2**) and mexicanin-E (**3**).

	(1)	(2)	(3)
H–1		2.62 ddd(2,3,9)	b
H–2	7.60 d(5)	7.71 dd(2,6)	7.75 dd(2,4.5)
H–3	6.18 d(5)	6.24 dd(3,6)	6.23 dd(2.5,4.5)
H–5	2.47 dd(2.5)		b
H–6	b	1.60 dd(4,12)	b
	b	1.75 dd(10.5,12)	b
H–7	3.19 m	3.57 br.ddd(4,4,10.5)	3.14 m
H–8	4.86 ddd(3,4,4)	4.66 ddd(3,4,4)	4.64 ddd(3,4,4)
H–13a	5.66 d(1.5)	5.62 d(1.5)	5.67 d(1.5)
H–13b	6.21 d(1.5)	6.16 d(1.5)	6.20 d(1.5)
H–14	1.16 d(5)	1.20 d(5)	1.19 d(6)

[a]Run at 250 MHz in $CDCl_3$ with Me_4Si as internal standard. Figures in parentheses are coupling constants in hertz.
[b]Signal obscured.

fused lactone since $J_{7,13a}=J_{7,13b}=1.5$ Hz. The configuration of the methyl group at C–10 was assigned on the basis of nmr data. If the C–10 methyl group were in the β-axial configuration, the H_α–8 would be expected to give rise to a dissimilar splitting pattern compared to that of **3** (table 1) due to difference in dihedral angles. This evidence led to the assignment of microhelenin-E (**1**) as the monohydroxylated mexicanin-E.

The position for the tertiary hydroxyl group was determined as follows. The ^{13}C-nmr spectral data (table 2) showed the downfield shifts of C–1 (Δδ +29.8), C–5 (Δδ +9.4) and C–10 (Δδ +6.3), and the upfield shifts of C–9 (Δδ −8.6) and C–14 (Δδ −4.3) in going from **3** to **1**. These observations of hydroxylation shift (α, β and γ-*gauche* effects, 11) indicated that the hydroxyl group of **1** must be located at C–1. Thus, the structure of microhelenin-E was established as **1**, i.e., the 1α-hydroxy-mexicanin-E.

Microhelenin-F (**2**, colorless gum) has the same molecular formula $C_{14}H_{16}O_4$ as **1**, as shown by a molecular ion peak at m/z 248.1045. Compound **2** gave ir, ^{1}H-nmr (table 1) and cd data[3] very similar to those of **1** and **3** indicating the presence of a *cis*-fused cyclopentenone moiety, an α-methylene-γ-lactone ring *cis*-fused at C–8, a secondary methyl and a tertiary hydroxyl groups. This evidence suggests that compound **2** is also a monohydroxylated mexicanin-E. A comparison of the chemical shifts of the ^{13}C-nmr spectra (table 2) between **2** and **3** clearly indicated that the tertiary hydroxyl group of **2** must be placed at C–5 position as in going from **3** to **2**, the carbon signals of C–5, C–1 and C–6 were shifted downfield by

[2]Compound **1** showed negative CE at λ max 332 nm and 263 nm, and positive CE at λ max 292 nm. Compound **3** exhibited negative CE at λ max 335 nm and 260 nm, and positive CE at λ max 300 nm.
[3]Compound **2** showed negative CE at λ max 335 nm and 265 nm, and positive CE at λ max 295 nm.

31.2, 7.8 and 5.1 ppm, respectively, whereas the carbon signals of C-3, C-7 and C-2 were shifted upfield and other carbon resonances remained almost unchanged. Microhelenin-F was therefore assigned as structure **2**, i.e., the 5α-hydroxymexicanin-E.

TABLE 2. ^{13}C-nmr spectra of microhelenin-E (**1**), microhelenin-F (**2**) and mexicanin-E (**3**) in $CDCl_3$.

Compd \ Carbon	1	2	3	4	5	6	7
1	83.3	163.6	132.6	206.4	57.5	31.9	44.8
$\Delta\delta$[a]	(+29.8)	(−2.8)		(−3.8)	(+9.4)		
2	61.3	164.9	130.4	210.9	80.0	36.7	39.6
$\Delta\delta$	(+7.8)	(−1.5)	(−2.5)		(+31.2)	(+5.1)	(−5.1)
3	53.5	166.4	132.9	210.2	48.1	31.4	44.8

Compd \ Carbon	8	9	10	11	12	13	14
1	78.4	29.9	33.6	140.6	168.9	122.0	17.2
$\Delta\delta$[a]		(−8.6)	(+6.3)				(−4.3)
2	78.3	38.0	27.4	141.5	170.7	122.0	21.5
$\Delta\delta$							
3	78.4	38.5	27.3	141.2	170.0	121.7	21.5

[a]$\Delta\delta = \delta_{C-(3)} - \delta_{C-(1)}$ and $\delta_{C-(3)} - \delta_{C-(2)}$.

Microhelenin-E (**1**) and -F (**2**) were tested for both *in vitro* and *in vivo* cytotoxic antileukemic activities against KB tissue culture cell and P-388 lymphocytic leukemia growth in BDF_1 male mice, respectively, according to a literature method (12). Compound **1** demonstrated significant cytotoxicity (ED_{50}=1.38 μg/mL) and antileukemic activity (T/C=166% at 8 mg/kg). However, compound **2** was found to be inactive in both systems.[4]

EXPERIMENTAL[5]

PLANT MATERIAL.—The *Helenium microcephalum* (Compositae) used was from a collection made in June 1972 in Burleson County, Texas, by Professor John J. Sperry of Texas A & M University. A voucher specimen (J. J. Sperry, No. 4020) is available for inspection at the Herbarium of the Department of Botany, University of North Carolina at Chapel Hill.

PRELIMINARY EXTRACTION.—The ground air-dried whole plant (7 kg) was exhaustively extracted with chloroform according to an exact procedure described in the literature (5)—to give 181 g of a dark brown gum.

[4]Microhelenin-F (**2**) showed an ED_{50}=11.0 μg/ml and a T/C=116% at 8 mg/kg in the same test.

[5]Melting points were determined on a Thomas-Hoover melting point apparatus and were uncorrected. Specific rotations were obtained on a Rudolph Autopol III automatic polarimeter (l=0.5 dm). Infrared (ir) spectra were recorded on a Perkin-Elmer 257 grating ir spectrometer. Proton nuclear magnetic resonance (pmr) spectra were determined on a Brucker 250 MHz spectrometer (Me_4Si as an internal standard). ^{13}C-nmr spectra were recorded on a Jeol FX-60 spectrometer functioning at 15.03 MHz. All nmr spectra were obtained with the use of the Fourier transform technique. The abbreviations s, d, t, q and m refer to singlet, doublet, triplet, quartet and multiplet, respectively. Mass spectra were determined on an A.E.I.MS-902 instrument at 70 eV using a direct inlet system. Circular dichroism (cd) spectrum was measured on a Cary model 60 spectrometer. Silica gel was used for both column (Merck silica gel 60, 70–230 mesh) and thin layer (Merck silica gel 60 F-254) chromatographies. Detection of components was made by spraying with 1% cerium sulfate—10% sulfuric acid solution followed by heating. High performance liquid chromatography (hplc) was performed on a Waters Associates Model ALC/GPC 244 Liquid Chromatograph with a Whatman Partisil M9 10/50 column.

ISOLATION OF MICROHELENIN-E (1) AND -F (2).—The brown gum was chromatographed on silica gel (2 kg) and eluted with chloroform, chloroform-acetone (3:1) and acetone. The first chloroform eluate afforded a gummy solid upon evaporation of the solvent. A half volume of this gummy solid was rechromatographed on silica gel (2.5 kg) and eluted with benzene, benzene-chloroform (1:1~1:3), chloroform, chloroform-acetone (1:1~1:3), acetone and acetone-methanol (1:1). After evaporation of the solvents, the chloroform and chloroform-acetone (1:1) eluates yielded both gummy solids [4.2 g (Fraction a) and 30 g (Fraction b), respectively].

Fraction a (3.8 g) was column chromatographed on Sephadex LH-20 (200 g, 5 x 100 cm) in chloroform (collected at 14 ml with a flow rate of 3 ml/min). Microhelenin-F (2, 80 mg) was isolated from fractions 61–70. Chromatography of Fraction b (30 g) performed by use of the same column, solvent and procedure described above for the purification of Fraction a; furnished fractions 85–100, from which crude microhelenin-E (1, 220 mg) was isolated. Further purification of 1 and 2 was achieved by means of high performance liquid chromatography [Whatman Partisil M9, particle size 10μ, L=500 mm, O.D.=12.8 mm, I.D.=9.4 mm, Detect: uv 254 nm, solvent: *n*-hexane–isopropanol (10:3), Flow rate: 4 ml/min in the recycle mode].

MICROHELENIN-E (1).—Microhelenin-E was recrystallized from benzene as colorless needles (78 mg, 0.001% yield): mp 135–136.5°; $[\alpha]^{25}D$+47.2° (C=1.2, chloroform); ms, *m/z* 248.1045 (M^+, $C_{14}H_{16}O_4$ requires 248.1048); ir ($CHCl_3$), ν max: 3450 (free OH), 1760, 1660 (α,β-unsaturated γ-lactone), 1710 and 1590 (α,β-unsaturated cyclopentenone) cm^{-1}. The ^1H-nmr and ^{13}C-nmr data have been shown in table 1 and 2, respectively.

MICROHELENIN-F (2).—This compound appeared as colorless gum (80 mg, 0.001% yield): $[\alpha]^{25}D$−33.3° (C=0.3, pyridine); ms, *m/z* 248.1045 (M^+, $C_{14}H_{16}O_4$ requires 248.1048); ir ($CHCl_3$), ν max: 3440 (free OH), 1760, 1665 (α,β-unsaturated γ-lactone), 1712 and 1585 (α,β-unsaturated cyclopentenone) cm^{-1}. The ^1H-nmr and ^{13}C-nmr data have been summarized in table 1 and 2, respectively.

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