ACKNOWLEDGMENTS

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Antitumor Agents XXXVI: Structural Elucidation of Sesquiterpene Lactones Microhelenins-A, B, and C, Microlenin Acetate, and Plenolin from *Helenium microcephalum*

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Abstract □ The antitumor sesquiterpene lactones microhelenins-A, B, and C, microlenin acetate, and plenolin were isolated from *Helenium microcephalum*. The structures and stereochemistry of these lactones were determined by physical methods as well as by chemical transformations and correlations. Microlenin acetate appears to be the first novel dimeric sesquiterpene lactone demonstrated to have significant antileukemic activity.

Keyphrases □ Antitumor agents—microhelenins, microlenin acetate, and plenolin, isolation from *Helenium microcephalum*, determination of structure and stereochemistry □ Microhelenins—isolation from *Helenium microcephalum*, determination of structure and stereochemistry □ Sesquiterpene lactones—microhelenins, microlenin acetate, and plenolin, isolation from *Helenium microcephalum*, determination of structure and stereochemistry

Search for an ample supply of helenalin for investigation into the relationship between the structure of sesquiterpene lactones and their cytotoxic antitumor activity led to the use of the plant *Helenium microcephalum*[1] (1). Preexamination of the whole plant extract revealed that the removal of helenalin left a mother liquor, which retained significant inhibitory activity against Walker 256 carcinosarcoma in rats and P-388 lymphocytic leukemia in mice. Further work with the chloroform extract resulted in the isolation[2] and structural determination of the new antitumor agents microhelenins-A (I) (2), B (VI) (3), C (VIII) (3), and D (mexicanin-E, XIV) (3) and microlenin[3] (4, 5), which were described in preliminary reports.

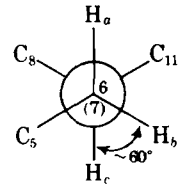
The purpose of this paper is to describe fully the isolation and structural elucidation of microhelenins-A, B, and C, the new dimeric antileukemic agent microlenin acetate[3] (X), and the companion pseudoguaianolide plenolin (VII) (7).

RESULTS AND DISCUSSION

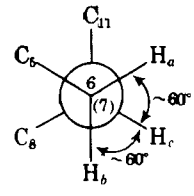
The final chloroform extract of the whole plant of *H. microcephalum* was chromatographed on silica gel using chloroform and chloroform–ethyl acetate (9:1) as the eluents. The initial chloroform eluate afforded a gum of several components. Further silica gel column chromatography and preparative TLC of this mixture led to the isolation of microhelenins-A, B, C, and D. Subsequent elution with chloroform–ethyl acetate (9:1) gave plenolin and microlenin as well as helenalin. Microlenin acetate was isolated from the mother liquor after the removal of helenalin.

Microhelenin-A (I)—Microhelenin-A (I), $C_{15}H_{18}O_4$ (high-resolution mass spectral and elemental analyses), mp 140–141°, $[\alpha]_D^{24}$ +89.0°, showed the partial structure A, with the lactone ring closed at C-7 and C-8 and the methylene group of CH_2OCH attached at C-5 on the basis of IR and NMR (Table I) evidence as reported previously (2).

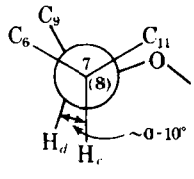
The circular dichroism and optical rotatory dispersion data of microhelenin-A are listed in Table II together with data for 2,3-dihydrohelenalin (II) (8), whose absolute configuration has been established. Similarities in the sign and magnitude of ketone Cotton effects indicated that I possessed the same *trans*-fused cyclopentanone ring system (C-1α, C-5β-CH_2O) as II. Determination of the *cis*-stereochemistry of the γ-lactone ring in I was based on the generalization that *cis*-fused lactones closed toward C-8, exhibiting a positive lactone Cotton effect, as exemplified by II (9, 10). In addition, Samek's rule (11) ($J_{7,13}$ *trans*-lactone $\geq 3 \geq J_{7,13}$ *cis*-lactone), which generally has been applicable to guaianolides, also indicated that I contains a *cis*-fused lactone since $J_{7,13e}$ = 2.5–3.0 and $J_{7,13f}$ = 2.0–3.0[4]. This evidence led to postulation of possible conformations of the seven-membered ring of I (C and D).



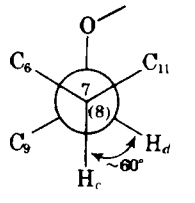
C: boat and half-chair



D: chair



E: boat



F: half-chair

[1] The constituents of *H. microcephalum* were examined previously and reported to contain helenalin in good yield (1).

[2] Helenalin also was isolated from this chloroform extract.

[3] Microhelenins-A, B, C, and D and microlenin showed significant (T/C ≥ 125%) inhibitory activity against Walker 256 carcinosarcoma in rats at T/C = 148, 138, 159, 144, and 172% at 2.5 mg/kg, respectively. Microlenin and microlenin acetate demonstrated significant (T/C ≥ 120%) inhibitory activity against P-388 lymphocytic leukemia in mice at T/C = 167 and 147% at 12.5 mg/kg, respectively, according to a literature method (6). Plenolin showed T/C = 138% at 25 mg/kg in the P-388 screen.

[4] The coupling constants of $J_{7,13}$ were changed by the conditions of the NMR instrument (XL-100), as shown in the data.

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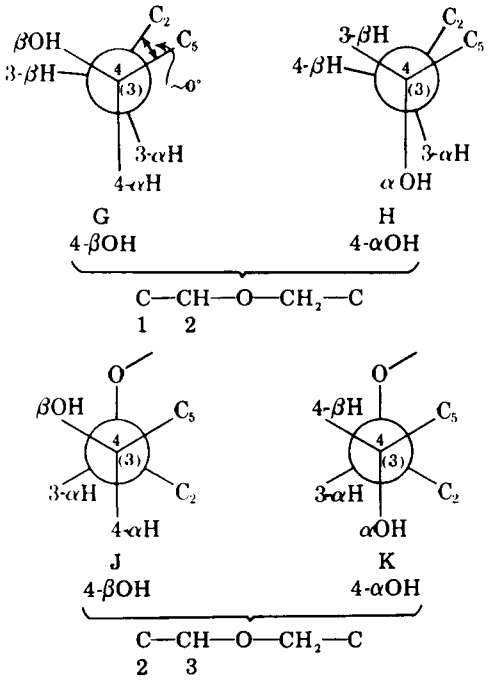
Table I—Decoupling Experiment Data for IX*b* in PMR Spectrum[a]

Changed Signal Position	Irradiation Position: C-11 CH_3 (δ 1.47)	H-7 (δ 2.74)	H-11 (δ 2.74)	H-8 (δ 4.71)	H-6 (δ 5.38)
C-11 CH_3			d, J = 7.5 ↓ s		
H-7			br dd, J = 6.0 and 10.0 ↓ d, $J_{7,8}$ = 6.0	br dd, J = 6.0 and 10.0 ↓ d, $J_{7,11}$ = 10.0	br dd, J = 6.0 and 10.0 ↓ sharp dd, and J = 6.0 and 10.0
H-11	dq, J = 7.5 and 11.0 ↓ d, $J_{7,11}$ = 10.0	dq, J = 7.5 and 11.0 ↓ d, $J_{11,13}$ = 7.5			
H-8		br t ↓ m			
H-6		br s, $W_{1/2}$ = 2.5 ↓ sharp s			

[a] Values are expressed in parts per million. Multiplicities are indicated by the usual symbols: d, doublet; m, multiplet whose center is given; br s, broad singlet; br t, broad triplet; dd, doublet of doublets; and dq, doublet of quartets. The values are in Hertz. Measurements were recorded with a 100-MHz instrument and were taken in deuterochloroform.



The conformation of the seven-membered ring was determined by a series of INDOR[5] experiments. The magnitude of $J_{6,7}$ determined the relative configuration at C-6 (H_a) and C-7 (H_c); hence, if the configuration of H_c were established as α, then the configurations for H_a and H_b at C-6 would be known accordingly. When the quartet lines of H_a (δ 1.67) were monitored, they gave the INDOR signals arising from H_b at $\delta \sim$ 2.30–2.50 (2). The J values observed between H_a and H_c (J = 13.0 Hz) clearly indicated a diaxial relationship between H_a and H_c, and the value between H_b and H_c (J = 3.0 Hz) indicated a dihedral angle of ~60°, as illustrated in C. Consequently, the conformation of I was assigned the boat or half-chair form.

In addition, the coupling constant ($J_{7,8}$ = 9.0 Hz) between H_c and H_d indicated an eclipsed relationship between H_c and H_d, as illustrated in E, and not a dihedral angle of ~60°, as illustrated in the half-chair form F. Thus, the boat form shown in B was the preferred conformation of the seven-membered ring of I. The linkage position of the remaining methine proton in CH_2OCH was determined as follows. The physical data of I suggested that the methine proton in CH_2OCH could be placed only at C-2 or C-3; in this case, the possible configuration relationships between C-3 and C-4 could be shown as G, H, J, and K (Structure B).

[5] Internal Nuclear Double Resonance.

Treatment of I with sodium borohydride afforded a monohydroxy 11,13-dihydro derivative (III*a*) in which the methylene group of CH_2OCH was linked at C-5 on the basis of spectral evidence (2).

The protons at C-2 and C-4 appeared as a multiplet at δ 4.0. However, the NMR spectrum of III*a* measured in deuterated chloroform–benzene (1:1) showed the presence of H-2 as a multiplet at δ 3.77 and of H-4 as a doublet of doublets of doublets at δ 3.49 (J = 2.0, 4.0, and 10.0 Hz), which indicated that the methine proton of CH_2OCH was placed at C-2, as illustrated in G.

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 C-2 β-oxygen group would be expected to deshield the C-10 β-methyl group (δ 1.34), as was shown for bipinantin (IV) (12). On the other hand, a C-2 α-hydroxyl group, as found in ivoxathin (V) (13), would be expected to have little or no effect on it (δ 1.11). In addition, a positive Nuclear Overhauser Effect (NOE) between a C-10 β-methyl group and the 5β-axial proton (H_h) should be observed.

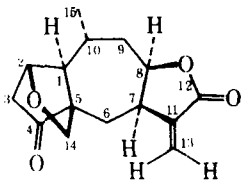
The C-10 methyl group of I was observed as a doublet at δ 1.17 and showed no NOE in the NMR spectrum. Therefore, the C-10 methyl group was assigned to an α-configuration and thus gave the complete Structure B for microhelenin-A. The high-resolution mass spectral data of I shown in Scheme I supported this structural assignment.

Microhelenins-B (VI) and C (VIII)—Microhelenin-B (VI), $C_{20}H_{28}O_5$ (high-resolution mass spectrum), mp 111–113°, $[\alpha]_D^{28}$ −84.91°, was recrystallized as colorless needles from chloroform. The IR (tetrachloromethane) and NMR spectra (Table III) showed the presence of an α,β-unsaturated cyclopentenone and an α-methyl-γ-lactone, as found in plenolin (VII) (7) as described previously (3).

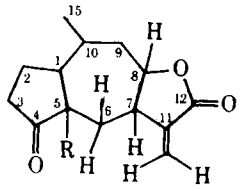
The remaining two oxygen atoms of the empirical formula were assigned as part of a five-carbon saturated ester side chain (IR bands at 1741, 1230, and 1190 cm^{-1}), as evidenced by the facile loss of a $C_5H_{10}O_2$ fragment and the appearance of a $C_5H_9O^+$ ion as the base peak in the mass spectrum (Scheme II). The nature of this ester side chain was revealed by the NMR spectrum, which had the same characteristic peaks of a 2-methylbutanoate, as found in tetrahydrobrevilin-A (IX*a*) (14). Based on the physical data, Structure VI was postulated for microhelenin-B. The attachment of the 2-methylbutanoyl side chain at C-6 (δ 5.50, br s, $W_{1/2}$ = 3.0 Hz, H at C-6) and the closing of the lactone ring at C-8 (δ 4.80, br t-like, J = 6.0 Hz, H at C-8) were based on comparable substitution patterns found in other pseudoguaianolides. The high-resolution mass spectral data supported the structural assignment (Scheme II).

Microhelenin-C (VIII), $C_{20}H_{26}O_5$ (high-resolution mass spectrum), $[\alpha]_D^{23}$ −85.0°, was isolated in small quantity as a gum by silica gel column chromatography and preparative TLC. The IR (tetrachloromethane) and NMR spectra (Table III) indicated the presence of an α-methyl-γ-lactone ring, an α,β-unsaturated cyclopentenone of the type found in VI and VII, and a tiglate[6] ester moiety as reported previously (3). In addition,

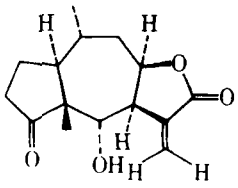
[6] In general, the βH and βCH_3 protons in methyl tiglate were observed at δ 6.73 and 1.73, respectively, and the protons in methyl tiglate were observed at δ 5.98 and 1.97, respectively (15). The NMR spectrum of VIII indicated the presence of a tiglate ester group, as shown in Table III.



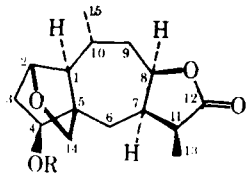
I



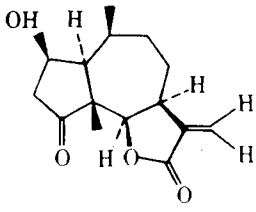
A



II

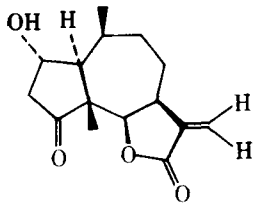


IIIa: R = H
IIIb: R = $COCH_3$



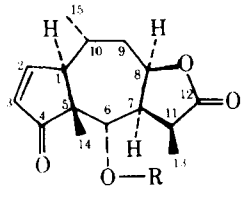
IV

NMR ($CDCl_3$):
δ 1.34 (d, 10-CH_3)

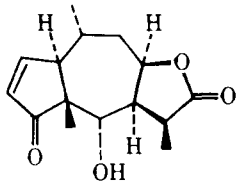


V

NMR ($CDCl_3$):
δ 1.11 (d, 10-CH_3)

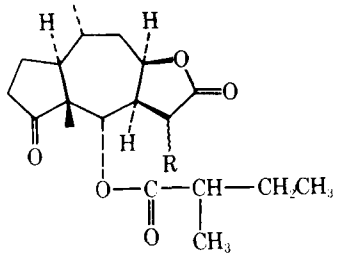


VI: R = $-\overset{1'}{\underset{\underset{O}{\|}}{C}}-\overset{2'}{\underset{\underset{CH_3}{|}}{CH}}-CH_2CH_3$

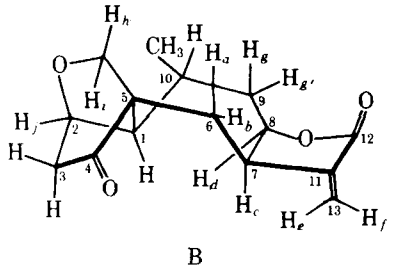


VII

VIII: R = $-\underset{\underset{O}{\|}}{C}-\underset{\underset{CH_3}{|}}{C}=C\overset{H}{\underset{CH_3}{}}$



IXa: R = αCH$_3$
IXb: R = βCH$_3$

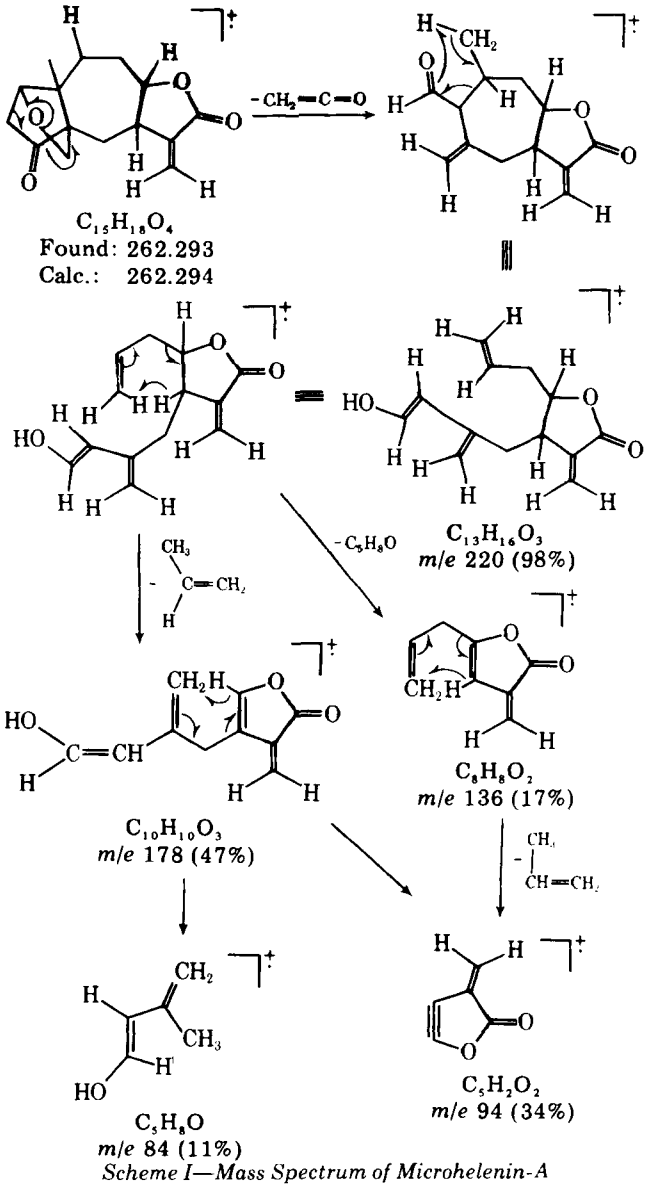


B

the PMR spectrum showed the presence of two methyl signals [δ 1.05 (s, 5-CH_3) and 1.25 (d, J = 6.0 Hz, 10-CH_3)]. In accordance with these deductions, the high-resolution mass spectrum exhibited diagnostically important peaks at m/e 346.1782 ($C_{20}H_{26}O_5$, M^+), 246.1252 ($M^+ - C_5H_8O_2$, base peak), 123.0808 ($C_8H_{11}O^+$), and 83.0494 ($C_5H_7O^+$). Consequently, the structure of microhelenin-C was formulated as VIII, and the stereochemistry was established by direct comparison (TLC and superimposable IR, NMR, and mass spectra) with the synthetic plenolin tiglate prepared from plenolin (VII) and tigloyl chloride in pyridine-benzene.

Catalytic hydrogenation of VI and VIII with 10% palladium-on-carbon in ethanol yielded the same amorphous compound (IX*b*). The IR, PMR, and mass spectra reflected changes resulting from saturation of a cyclopentenone chromophore and a tigloyl side chain. In addition, the PMR spectrum (Table III) was strikingly similar to that of tetrahydrobrevilin-A (IX*a*), which differed only in the stereochemistry of the methyl group at C-11. Thus, the conformations of the seven-membered ring and the configurations of 11-CH_3 could be described as shown in L–N.

The complete assignments of protons for C-6, C-7, C-8, and C-11 of IX*b* were achieved by extensive decoupling experiments (Table I). If the usual assumption is made that the C-7–C-11 bond is equatorial and β as in all sesquiterpene lactones of documented stereochemistry, then the observed large value of $J_{7,11}$ requires that H-11 be *cis* to H-7 and α and the small value of $J_{6,7}$ requires that H-6 be *cis* to H-7 and β. Thus, the lactone ring must be *cis*-fused and the seven-membered ring must be in the chair conformation, as shown in L. In addition, the stereochemistry of tetrahydromicrohelenins-B and C was confirmed to be identical to the



Scheme I—Mass Spectrum of Microhelenin-A

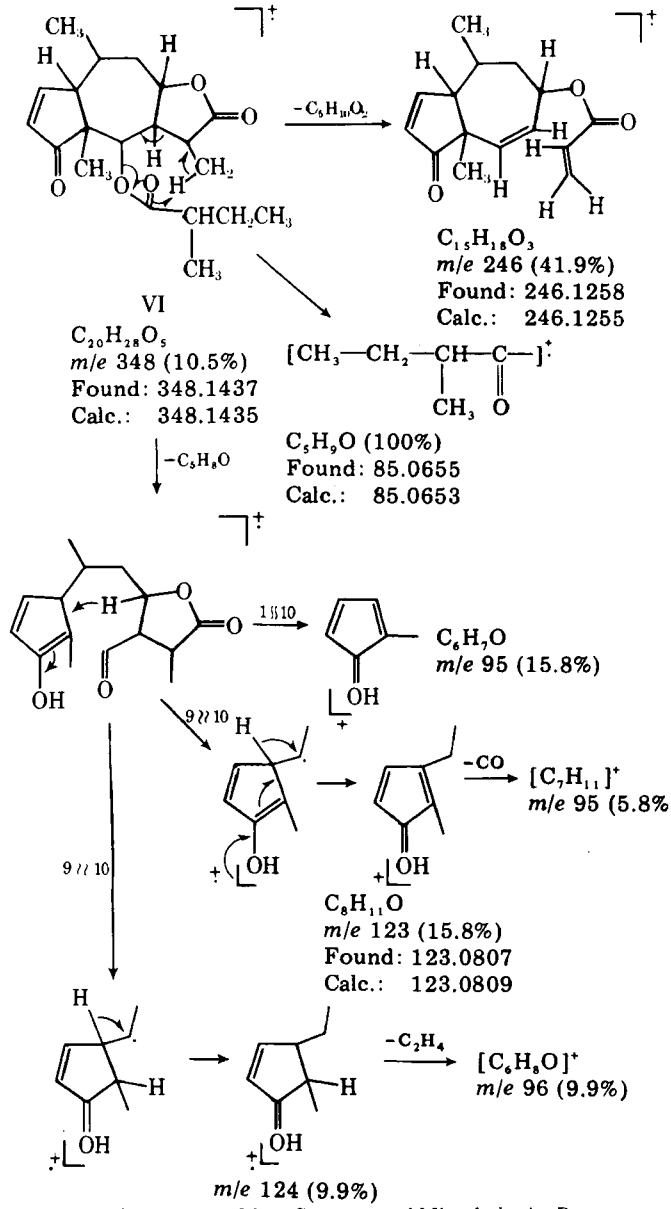
Table II—Optical Rotatory Dispersion and Circular Dichroism Curves of Microhelenin-A (I) and 2,3-Dihydrohelenalin (II)[a]

Compound	Optical Rotatory Dispersion: First Extreme nm	Φ	Second Extreme nm	Φ	Amplitude (a)	Circular Dichroism: Ketone λ_{max}, nm	θ	Lactone λ_{max}, nm	θ
I	313	+5633	277	−4821	+104.5	297	+7598	253	−3144
II	315	+5016	283	−1457	+63.41	300	+5280	262	−3749

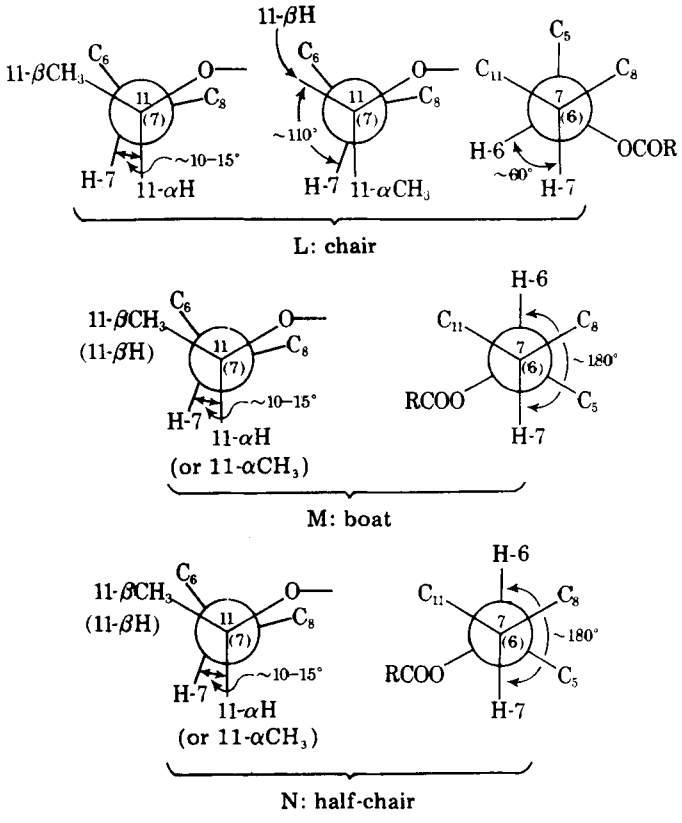
[a] Determined on a Cary 60 recording spectrometer in methanol (c, 0.1).

hydrogenation product prepared from plenolin tiglate by direct comparison of TLC results and IR and NMR spectra. Thus, the stereochemistry of tetrahydromicrohelenins-B and C was established as IX*b*. The foregoing evidence led to the establishment of the structures of microhelenins-B and C as VI and VIII, respectively.

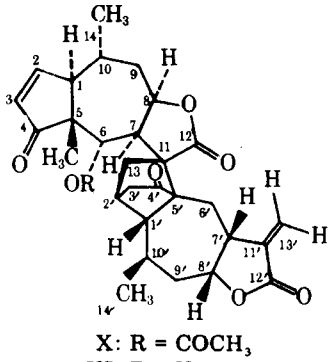
Microlenin Acetate (X)—Microlenin acetate (X), $C_{31}H_{36}O_8$, *m/e* 536 (M^+), mp 289–291° dec., was isolated from the mother liquor after the removal of helenalin and recrystallized as colorless needles from chloroform–ether. In the PMR spectrum of X, the presence of one acetyl singlet (δ 1.97), one methyl singlet (δ 0.94), and two methyl doublets [δ 1.18 and 1.23 (d, 3H each, J = 7.0 Hz)] coupled with the characteristic signals corresponding to one cyclopentenone [δ 7.67 (dd, J = 2.0 and 6.0 Hz) and 6.06 (dd, J = 3.0 and 6.0 Hz)] and one α-methylene-γ-lactone moiety [δ 6.28 (d, J = 3.0 Hz) and 5.52 (d, J = 3.0 Hz)] suggested an acetylated dimeric sesquiterpene lactone possessing a cyclopentenone and an α-methylene-γ-lactone for the structure of X. Final identification of X with an acetate of microlenin (XI), prepared by acetylation of XI with acetic anhydride–pyridine, was established by direct comparison (IR, NMR, and mass spectra).

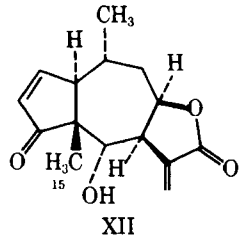


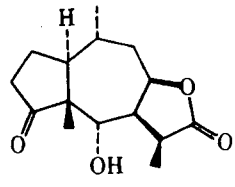
Scheme II—Mass Spectrum of Microhelenin-B



Plenolin (VII)—Plenolin (VII), $C_{15}H_{20}O_4$, *m/e* 264 (M^+), mp 222–224°, was isolated from the chloroform–ethyl acetate (9:1) eluate and recrystallized as colorless needles from chloroform. The IR and NMR







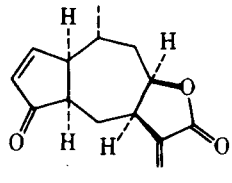


Table III—PMR Spectra of Microhelenins-A (I), B (VI), and C (VIII) and IX*b* [a]

Assignment	I	VI[b]	VIII	IX*b*
H-2	δ 4.53 (m)	δ 7.79 (dd, 2.3 and 6.0)	δ 7.76 (dd, 2.0 and 6.0)	
H-3		δ 6.14 (dd, 3.0 and 6.0)	δ 6.06 (dd, 3.0 and 6.0)	
H-6	δ 1.67 (dd, 13.0 and 15.0, H_a)	δ 5.50 (s-like, $W_{1/2}$ = 3.0)	δ 5.46 (s-like, $W_{1/2}$ = 3.0)	δ 5.38 (br s)
	δ 2.44 (dd, 3.0 and 15.0, H_b)			
H-7	δ 3.12 (m)			δ 2.74 (dd, 6.0 and 10.0)
H-8	δ 4.77 (ddd, 4.0, 9.0, and 11.0)	δ 4.80 (m)	δ 4.78 (m)	δ 4.71 (br t-like, 6.0)
H-11				δ 3.03 (dq, 7.5 and 10.0)
H-13e	δ 5.77 (d, 3.0)			
H-13f	δ 6.34 (d, 3.0)			
H-15	δ 3.64 and 3.85 (*AB*q, 9.0)			
5-CH_3		δ 1.08 (s)	δ 1.05 (s)	δ 0.84
10-CH_3	δ 1.17 (d, 7)	δ 1.28 (d, 6.0)	δ 1.25 (d, 6.0)	δ 1.06 (d, 7.0)
11-CH_3		δ 1.55 (d, 6.0)	δ 1.50 (d, 6.0)	δ 1.47 (d, 7.5)
2′-CH_3		δ 1.08 (d, 7.5)	δ 1.73 (br s)	
3′-CH_3			δ 1.74 (br d, 7.0)	
H-3′			δ 6.64 (m)	
2′-CH_3		δ 1.08 (d, 7.5)	δ 1.73 (br s)	δ 1.06 (d, 7.0)
3′-CH_3		—[c]	δ 1.74 (br d, 7.0)	—[c]
H-3′		—[c]	δ 6.64 (m)	—[c]

[a] Values are in parts per million. Multiplicities are indicated by the usual symbols: d, doublet; m, multiplet whose center is given; and br, slightly broadened singlet. Unmarked signals are singlets. Figures in parentheses are coupling constants in Hertz. Measurements were recorded with a 100-MHz instrument and were taken in deuterochloroform. [b] Measured at 60 MHz (in deuterochloroform). [c] Obscured signal.

spectra of this compound were identical with those of synthetic dihydrohelenalin prepared by catalytic hydrogenation of helenalin. To confirm further the structure of VII, its identity with natural plenolin, isolated from Florida *Helenium autumnale* (15), was established by direct comparison (TLC and superimposable IR, NMR, and mass spectra).

EXPERIMENTAL[7]

Isolation of Microhelenins-A (I), B (VI), C (VIII), and D (XIV), Microlenin Acetate (X), and Plenolin (VII) from *H. microcephalum*.—The *H. microcephalum* (Compositae) used was from a collection made in June 1972 in Burleson County, Tex[8]. The ground, air-dried, whole plant material (7 kg) was extracted exhaustively with chloroform and worked up in the usual manner (16), affording 181 g of a dark-brown gum. The gum (146 g) was chromatographed on silica gel (10 × 70 cm) with chloroform, chloroform–ethyl acetate (9:1), and acetone. Fractions of 250 ml were collected (Fractions a, b, and c) and examined by TLC.

The first chloroform eluate (fractions 7–15 = Fraction a) gave a gummy solid (10 g) upon evaporation of the solvent. The subsequent chloroform eluate (fractions 16–25 = Fraction b) yielded a gum which, upon chromatography on silica gel [200 g, eluted with benzene–chloroform (1:1) and collected at 75 ml/fraction], gave 0.2 g of microhelenin-A upon recrystallization of fractions 13–32 with ether. Recrystallization with ether–tetrachloromethane of the mother liquor after the removal of microhelenin-A afforded 0.2 g of microhelenin-D. The chloroform–ethyl acetate (9:1) eluate (fractions 50 and 51 = Fraction c) yielded helenalin (XII) and plenolin (VII, 80 mg) in addition to a gummy solid (1 g), which was recrystallized from chloroform–ether to give 400 mg of microlenin (XI) as colorless needles. Microlenin acetate (X, 28 mg) was isolated from the mother liquor after the removal of helenalin.

The first chloroform eluate (Fraction a, 10 g) was rechromatographed on silica gel (200 g) with benzene–acetone (25:1) (collected at 20 ml/fraction). Fractions 50–60, after evaporation of the solvent, left a semisolid residue (318 mg). This residue was purified by preparative TLC [benzene–chloroform–acetone (4:4:1), UV detection] to yield a mixture of microhelenins-B and C (205 mg) as indicated by NMR and mass spectra. Further purification of this mixture was achieved by a second preparative TLC procedure [silica gel impregnated with silver nitrate, chloroform–ethyl acetate (3:1), developed twice and detected with UV light] to give 109 mg of microhelenin-B (VI) and 50 mg of microhelenin-C (VIII). Fractions 81–180 gave microhelenin-D (mexicanin-E) (1.8 g) after recrystallization from ether–tetrachloromethane. Fractions 190–250 yielded microhelenin-A (30 mg) upon recrystallization from chloroform.

Microhelenin-A (I)—The analytical data for I were: mp 140–141°, $[\alpha]_D^{24}$ +89.0° (*c*, 1.00 in methanol), C, 68.47; H, 6.88 ($C_{15}H_{18}O_4$ requires C, 68.68; H, 6.92); IR (tetrachloromethane): no hydroxyl group, 1772, 1665 (α-methylene-γ-lactone), and 1758 (cyclopentanone) cm^{-1}; mass spectrum: *m/e* (%) 262.293 (M^+, 100) (calc. 262.294), 220 [M^+ − 42 (CH_2=CO), 98], 178 [M^+ − (42 + CH_3CH=CH_2), 47], 136 [M^+ − (42 + 84), 17], 94 ($C_5H_5O_2^+$, 34), and 84 ($C_5H_8O^+$, 11). The PMR data (100 MHz, deuterochloroform) are shown in Table III.

Microhelenin-B (VI)—The analytical data for VI were: mp 111–113°, $[\alpha]_D^{28}$ −84.91° (*c*, 1.75 in methanol); IR (tetrachloromethane): 1782 (γ-lactone), 1741 (ester), 1726, and 1586 (cyclopentenone) cm^{-1}; mass spectrum: *m/e* (%) 348.1937 (M^+, 6.9) ($C_{20}H_{28}O_5$ requires 348.1935), 246.1258 (M^+ − $C_5H_{10}O_2$, 41.9) ($C_{15}H_{18}O_3$ requires 246.1255), 123.0807 ($C_8H_{11}O^+$, 15.8) ($C_8H_{11}O$ requires 123.0809), and 85.0655 [$CH_3CH_2CH(CH_3)C$=$O)^+$, 100] (C_5H_9O requires 85.0653). The PMR data (60 MHz, deuterochloroform) are shown in Table III.

Microhelenin-C (VIII)—The analytical data for VIII (a gum) were: $[\alpha]_D^{23}$ −85.0° (*c*, 1.30 in methanol); IR (tetrachloromethane): 1782 (α-methyl-γ-lactone), 1725, 1583 (cyclopentenone), 1725, 1651, 1260, and 1182 (tigloyl ester) cm^{-1}; mass spectrum: *m/e* (%) 346.1782 (M^+, 11.8) ($C_{20}H_{26}O_5$ requires 346.1779), 246.1252 (M^+ − $C_5H_8O_2$, 100) ($C_{15}H_{18}O_3$ requires 246.1255), and 83.0494 [$CH_3CH_2CH(CH_3)C$=O^+, 98.1] (C_5H_7O requires 83.0496). The PMR data (60 Hz, deuterochloroform) are shown in Table III.

Sodium Borohydride Reduction of Microhelenin-A (I)—To a solution of I (40 mg) in methanol (2 ml) was added sodium borohydride (20 mg). The total mixture was kept stirring at room temperature for 25 min, treated with acetone (5 ml) and methanol (3 ml), stirred further for 10 min, and worked up in the usual manner. The reaction product was purified by preparative TLC [benzene–methanol (20:2), detection with iodine] to give a monohydroxy dihydro derivative (III*a*) (15 mg), mp 138–140° (chloroform); IR (chloroform): 3590 (OH) and 1762 (γ-lactone) cm^{-1}; mass spectrum: *m/e* (%) 266.1518 (M^+, 9.8) ($C_{15}H_{22}O_4$ requires 266.1518) and 248 (M^+ − H_2O, 4.5); PMR (100 MHz, deuterochloroform): δ 1.07 (d, 3H, *J* = 7.0 Hz, 10-CH_3), 1.18 (d, 3H, *J* = 7.5 Hz, 11-CH_3), 2.84 (dq, 1H, *J* = 7.5 and 10.0 Hz, H-11), 3.50 (dd, 1H, *J* = 2.0 and 8.0 Hz, C-5 CH_2O), 4.18 (d, 1H, *J* = 8.0 Hz, 1H, C-5 CH_2O), 4.0 (m, 2H, H-2 and H-4), and 4.78 (m, 1H, H-8); PMR (100 MHz, deuterated chloroform and benzene, deuterated water treatment): δ 0.81 and 0.99 (d, 3H each, *J* = 7.0 Hz, 10-CH_3 and 11-CH_3), 3.23 (dd, 1H, *J* = 2.0 and 8.0 Hz, C-5 CH_2O), 3.49 (ddd, 1H, *J* = 2.0, 4.0, and 10.0 Hz, H-4), 3.77 (m, 1H, H-2), 3.98 (d, 1H, *J* = 8.0 Hz, C-5 CH_2O), and 4.29 (m, 1H, H-8).

Acetylation of III*a*—Acetylation of III*a* (7 mg) with acetic anhydride (0.5 ml) and pyridine (0.5 ml) at room temperature for 20 hr followed by the usual workup yielded a product (5 mg). This product was purified

[7] Melting points were determined on a Thomas-Hoover melting-point apparatus and are uncorrected. Specific rotations were on a Perkin-Elmer model 141 polarimeter (1 = 1 dm). IR spectra were recorded with a Perkin-Elmer model 257 grating IR spectrometer. The PMR spectrum was determined with either a Jeolco C60 HL or a Varian XL-100 NMR spectrometer (tetramethylsilane as the internal standard). Mass spectra were determined on an AEI MS-902 instrument at 70 ev using a direct-inlet system. Optical rotatory dispersion and circular dichroism spectra were measured on a Cary model 60 spectrometer. Silica gel was used for column chromatography (Mallinckrodt CC-7, 200–325 mesh) and for TLC (Merck silica gel G). Detection of components was made by spraying with 1% cerium sulfate–10% sulfuric acid solution followed by heating. Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga.

[8] Dr. John J. Sperry, Texas A & M University, collected and identified the plant material. A voucher specimen (J. J. Sperry, No. 4020) is available for inspection at the Herbarium, Department of Botany, University of North Carolina at Chapel Hill.

with preparative TLC [chloroform–methanol (40:1), detection with iodine] to give an acetylated derivative (III*b*, 3.5 mg) of III*a*. The analytical data for III*b* were: mp 135–138° (chloroform); mass spectrum: *m/e* (%) 308.1620 (M^+, 100) ($C_{17}H_{24}O_5$ requires 308.1624) and 248 (M^+ – CH_3COOH, 33); PMR (100 MHz, deuterochloroform): δ 2.14 (s, 3H, C-4 $OCOCH_3$), 4.09 (m, 1H, H-2), 4.7, and 5.1 (m, 2H, H-4 and H-8).

Catalytic Hydrogenation of Microhelenin-C (VIII)—A solution of VIII (50 mg) in ethanol (8 ml) was shaken with hydrogen at room temperature (22°) and atmospheric pressure in the presence of 10% palladium-on-charcoal (50 mg). The reaction mixture was filtered after the initial rapid absorption slowed. Removal of ethanol *in vacuo* resulted in a gum (49 mg), which was purified by preparative TLC [benzene–chloroform–acetone (4:4:1), detection with iodine] to give an amorphous compound (IX*b*, 36 mg) from acetone; IR (tetrachloromethane): 1782 (α-methyl-γ-lactone), 1749, and 1740 (sh) (ester and cyclopentanone) cm^{-1}; mass spectrum: *m/e* (%) 350.2091 (M^+, 7.0) ($C_{20}H_{20}O_5$ requires 350.2093), 248 [M^+ – $CH_3CH_2CH(CH_3)COOH$, 58.3], 85 $[CH_3CH_2CH_2C{=}O)^+$, 58.3] and 57 ($CH_3CH_2C^+HCH_3$, 100); PMR (100 MHz, deuterochloroform): δ 0.84 (s, 3H, 5-CH_3), 1.06 (d, 6H, J = 7.0 Hz, 10-CH_3 and 2′-CH_3), 1.47 (d, 3H, J = 7.5 Hz, 11-CH_3), 2.74 (dd, 1H, J = 6.0 and 10.0 Hz, H-7), 3.03 (dq, 1H, J = 7.5 and 10.0 Hz, H-11), 4.71 (br t-like, 1H, J = 6.0 Hz, H-8), and 5.38 (br s, 1H, H-6). The methyl triplet of the side chain was submerged in the methyl and methylene envelope.

Catalytic Hydrogenation of Microhelenin-B (VI)—The hydrogenation of VI was repeated under the described conditions. The product was identical to IX*b* according to TLC (three solvent systems), IR (tetrachloromethane), and PMR spectra (deuterochloroform).

Synthesis of Microhelenin-C (VIII) from Plenolin and Tigloyl Chloride—A solution of plenolin (120 mg) in dry benzene (2 ml) and dry pyridine (1 ml) was treated with tigloyl chloride (0.2 ml). The solution was heated under reflux for 3 hr. Evaporation to dryness under reduced pressure left a solid residue, which was suspended in 5% sodium bicarbonate, extracted with ether, and worked up in the usual manner. The product obtained from the ether extract was purified by silica gel (40 g, eluted with chloroform) column chromatography to give plenolin tiglate (54 mg) as a gum. This gum was identified as VIII by direct comparison with the material isolated previously from this plant; IR (tetrachloromethane): 1781 (α-methyl-γ-lactone), 1725, 1583 (cyclopentenone), 1725, 1651, 1262, and 1182 (tigloyl ester) cm^{-1}; PMR (60 MHz, deuterochloroform): δ 1.05 (s, 3H, 5-CH_3), 1.23 (d, 3H, J = 6.0 Hz, 10-CH_3), 1.51 (d, 3H, J = 6.0 Hz, 11-CH_3), 1.70 (br s, 3H, 2′-CH_3), 1.71 (br d, 3H, J = 6.5 Hz, 3′-CH_3), 4.75 (m, 1H, H-8), 5.47 (s-like, 1H, $W_{1/2}$ = 3.0 Hz, H-6), 6.04 (dd, 1H, J = 3.0 and 6.0 Hz, H-3), 6.65 (m, 1H, H-3′), and 7.70 (dd, 1H, J = 2.0 and 6.0 Hz, H-2).

Catalytic Hydrogenation of Plenolin Tiglate—A solution of the plenolin tiglate (45 mg) in ethanol (8 ml) was hydrogenated with 10% palladium-on-charcoal until hydrogen absorption ceased and then was treated as described. The product was purified by preparative TLC [benzene–chloroform–acetone (4:4:1), detection with iodine] to give an amorphous compound (30 mg) from acetone. The compound was identical with IX*b*, which was obtained previously by hydrogenation of VI and VIII. The IR spectra were superimposable, and the TLC behavior was identical; PMR (60 MHz, deuterochloroform): δ 0.83 (s, 3H, 5-CH_3), 1.05 (d, 3H, J = 6.75 Hz, 10-CH_3), and 1.46 (d, 1H, J = 6.75 Hz, H-10).

Microlenin Acetate (X)—The analytical data for X were: mp 289–291° dec., $[\alpha]_D^{20}$ +47.9° (*c*, 1.90 in methanol); IR (chloroform): no hydroxyl group, 1757 (γ-lactone), 1741, 1662 (α-methylene-γ-lactone), 1715, and 1580 (cyclopentenone) cm^{-1}; NMR (100 MHz, deuterochloroform): δ 0.94 (s, 3H, 5-CH_3), 1.18 and 1.23 (d, 3H each, J = 7.0 Hz, 10-CH_3 and 10′-CH_3), 1.97 (s, 3H, C-6 $OCOCH_3$), 2.48 (d, 1H, J = 8.0 Hz, H-7), 3.29 (m, 1H, H-7′), 4.71 (m, 1H, H-8′), 4.95 (m, 1H, H-8), 5.52 and 6.28 (d, 1H each, J = 3.0 Hz, H-13′), 5.62 (s, 1H, H-6), 6.06 (dd, 1H, J = 3.0 and 6.0 Hz, H-3), and 7.67 (dd, 1H, J = 2.0 and 6.0 Hz, H-2). The IR and NMR spectra of X were identical to those of microlenin acetate prepared from acetylation of microlenin (XI) with acetic anhydride in pyridine (5).

Plenolin (VII)—The analytical data for VII were: mp 222–224°; IR (chloroform): 3450 (hydroxyl), 1752 (γ-lactone), and 1690 (cyclopentenone) cm^{-1}; NMR (100 MHz, deuterochloroform): δ 0.96 (s, 3H, 5-CH_3), 1.21 (d, 3H, J = 7.0 Hz, 10-CH_3), 1.35 (d, 3H, J = 7.0 Hz, 11-CH_3), 4.40 (d, 1H, J = 4.0 Hz, H-6), 4.80 (m, 1H, H-8), 6.05 (dd, 1H, J = 3.0 and 6.0 Hz, H-3), and 7.70 (dd, 1H, J = 2.0 and 6.0 Hz, H-2); mass spectrum: *m/e* 264 (M^+).

Catalytic Hydrogenation of Helenalin (XII)—A solution of XII (60 mg) in ethyl acetate (25 ml) was hydrogenated with hydrogen at room temperature and atmospheric pressure in the presence of 5% palladium-on-carbon (25 mg). After the reaction mixture was filtered and the solvent was removed *in vacuo*, the residue was purified by column chromatography [silica gel, chloroform–ethyl acetate (9:1)] and preparative TLC [chloroform–ethyl acetate (1:1)] to give rise to the dihydro (23 mg, mp 222–224°) and the tetrahydro (XIII, 25 mg, mp 175–176°) derivatives.

The IR and NMR spectra of the dihydro derivative were identical to those of plenolin (VII) (7). The spectral data of the tetrahydro derivative were in accord with the structure of 2,3,11,13-tetrahydrohelenalin (XIII) (8, 17); mass spectrum: *m/e* 266 (M^+); IR (chloroform): 3580 (hydroxyl), 1750 (γ-lactone), and 1720 (cyclopentenone) cm^{-1}; NMR (deuterochloroform): δ 0.80 (s, 3H, 5-CH_3), 1.04 (d, 3H, J = 7.0 Hz, 10-CH_3), 1.36 (d, 3H, J = 7.0 Hz, 11-CH_3), 4.30 (br s, 1H, H-6), 4.75 (t-like, 1H, J = 6.0 Hz, H-8), and 2.95 (m, 2H, H-3).

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