ANTITUMOR AGENTS 37.[1] THE ISOLATION AND STRUCTURAL ELUCIDATION OF ISOHELENOL, A NEW ANTILEUKEMIC SESQUITERPENE LACTONE, AND ISOHELENALIN FROM *HELENIUM MICROCEPHALUM*

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ABSTRACT.—The known compound isohelenalin (**1**) was isolated from *Helenium microcephalum* and the previously reported structure confirmed on the basis of physicochemical data and chemical transformation. In addition, a new antileukemic sesquiterpene lactone, isohelenol (**2**), was isolated and its structure elucidated.

Search for ample supplies of helenalin for use in determining the relationship between structure and antitumor activity among sesquiterpene lactones led to the investigation of the plant *Helenium microcephalum*. This plant previously was reported to contain helenalin (**4**) in good yeields (1). Investigation of the minor constituents of this plant has led to the isolation of several new antitumor sesquiterpene lactones, microhelenins -A, -B, -C, microlenin and microlenin acetate[3] (2–5). In this paper, the isolation and structure elucidation of two more minor constituents, isohelenalin (**1**) and isohelenol (**2**), are reported.

Isohelenalin was isolated by column chromatography of the chloroform extract of the whole, dried plant. The same substance was previously isolated from the same plant by Büchi and Rosenthal (6). The structure assigned to this compound (**5**) was later modified by Herz and his associates, who synthesized isohelenalin by catalytic isomerization of helenalin (7). In both cases, little physical data ([α]D, mp, ir, and uv) was reported. Our work confirms the assignment of structure **1** for isohelenalin as reported by Herz and associates (7).

Isohelenalin (**1**) showed the presence of a cyclopentenone ring (ir bands at 1700, 1575 cm $^{-1}$ and ^{1}H-nmr doublet of doublets at δ 7.72 and 6.20), a secondary methyl (^{1}H-nmr doublet at δ 1.36), and an angular methyl (^{1}H-nmr singlet at δ 1.02) group—typical of helenalin-type sesquiterpene lactones. In addition, the ^{1}H-nmr showed the presence of a narrowly split vinyl methyl group at δ 1.96. The physical data suggested that isohelenalin was an isomer of helenalin with the double bond in conjugation with the γ-lactone endocyclic instead of exocyclic as in helenalin (**4**).

The stereochemistry of isohelenalin was assigned by two experiments. First, isohelenalin was oxidized by pyridinium chlorochromate to give **6**, which was identical to the compound synthesized by oxidation of helenalin. This confirmed that isohelenalin had the same stereochemistry as helenalin at C–1, C–5, C–8, and C–10. That left only the stereochemistry at C–6 to be assigned; this was accomplished by use of a NOE ^{1}H-nmr experiment. Irradiation of the signal for the

[1]Dedicated to the memory of the late Professor T. O. SOINE of the University of Minnesota. For Part 36, see S. A. ElGebaly, I. H. Hall, K. H. Lee, Y. Sumida, Y. Imakura and R. Y. Wu, *J. Pharm. Sci.*, in press.

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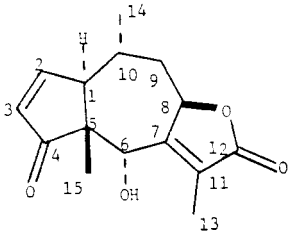

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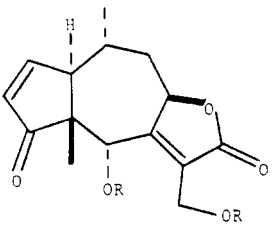
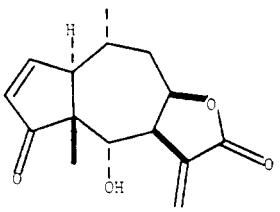


2, R=H
3, R=$COCH_3$



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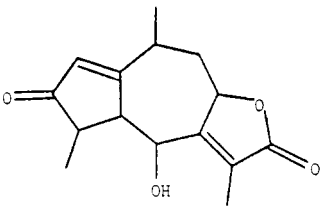

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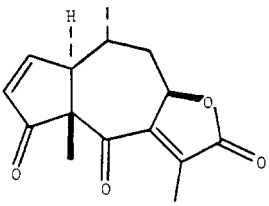

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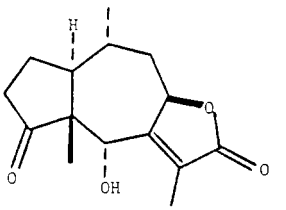

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angular methyl group (δ 0.84) of 2,3 dihydroisohelenalin (**7**), obtained by catalytic hydrogenation of **1** with platinum oxide in ethanol, resulted in an 8% increase in the signal for H–6. Thus, H–6 is β, which means the hydroxyl group at C–6 is α as in helenalin.

Isohelenol (**2**) was isolated from the more polar fractions of the plant extract. The ^{1}H-nmr spectrum was very similar to that of isohelenalin (table 1). It showed the characteristic doublet of doublets at δ 7.74 and δ 6.20 for the olefinic protons of the cyclopentenone, as well as the secondary and angular methyls at δ 1.36 and δ 1.02, respectively. Acetylation of isohelenol with acetic anhydride-pyridine gave a diacetate (**3**) (^{1}H-nmr showed two acetyl methyls at δ 2.10 and 1.96) which indicated isohelenol was a diol. The ^{13}C-nmr spectrum was comparable to those of helenalin and the helenalin portion of microlenin (5). In addition, it showed the presence of the endocyclic double bond (singlet peaks at δ 166.80 and 129.26). This evidence led to the assignment of the plane structure of isohelenol devoid of stereochemistry.

The stereochemistry of isohelenol was assigned on the basis of a NOE ^{1}H-nmr experiment. Irradiation of the angular methyl group at C–5 resulted in increases in the signals for the 11–CH_2 (9.4%) and the multiplet for H–10 and H–$9_{a,b}$ (15.4%). Drieding models of the possible structures of isohelenol gave only one structure **8** which fits this physical data. In addition, the similarities in the ^{1}H-nmr spectra

of isohelenalin and isohelenol, coupled with the biogenetic implications suggested by the co-occurrence of isohelenol (**2**) and isohelenalin (**1**) as well as helenalin (**4**), supported the assignment of the structure of isohelenol as **2**.

Isohelenol was tested for *in vivo* antitumor activity against P388 lymphocytic leukemia. A T/C value of 133% was seen at a dose of 12.5 mg/kg.[4] Further studies on the structure-activity relationships and mechanism of action among the isohelenol-related sesquiterpene lactones are currently in progress (9).

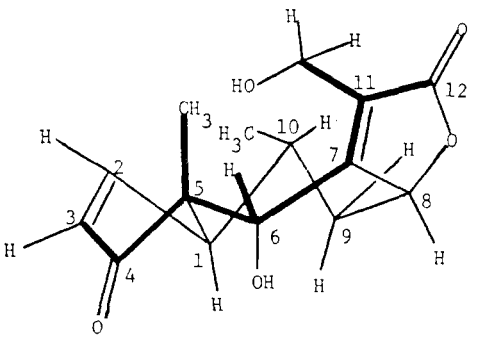

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TABLE 1. *Similarities in pmr spectra of isohelenalin* (**1**) *and isohelenol* (**2**).[a]

Assignment	Isohelenalin	Isohelenol
H-1	3.28 (m)	3.45 (m)
H-2	7.72 (dd, 2.0, 6.0)	7.74 (dd, 2.0, 6.0)
H-3	6.20 (dd, 3.0, 6.0)	6.20 (dd, 3.0, 6.0)
H-6	5.08 (br. s)	5.18 (br. s)
H-8	5.30 (m)	5.38 (m)
CH_3-5	1.02 (s)	1.02 (s)
CH_3-10	1.36 (d, 6.0)	1.36 (d, 6.0)

[a]Values are in parts per million, multiplicities are indicated by the usual symbols: s, singlet; d, doublet; m, multiplet whose center is given. Numbers in parenthesis are coupling constants in hertz.

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.

[4]*In vivo* activity was assayed by standard National Cancer Institute procedures described by literature 8. T/C values $\geq 125\%$ are considered significant.

[5]Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (ir) were recorded on a Perkin-Elmer 257 grating spectrophotometer. ^{1}H-nuclear magnetic resonance (^{1}H-nmr) spectra were recorded on a Varian XL-100 spectrometer and are given in parts per million (δ) downfield from an internal tetramethylsilane (TMS) standard. ^{13}C-nmr spectra were recorded on a Jeolco FX-100 spectrometer functioning at 25.20 MHz. 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. The abbreviation M+ refers to the molecular ion. Silica gel for column chromatography refers to Merck silica gel 60 (70–230 mesh). Silica gel for preparative thin layer chromatography (ptlc) refers to Analtech silica gel G (1000 microns). Compounds were visualized by ultraviolet light, iodine vapor, or by spraying with 1% $Ce(SO_4)_2$–10% H_2SO_4 solution followed by heating.

PRELIMINARY EXTRACTION.—The ground air-dried whole plant (7 kg) was exhaustively extracted with chloroform and evaporated *in vacuo* to give a crude oil. This oil was dissolved in methanol-water (2:1) and extracted several times with hexane. The aqueous layer was concentrated and then extracted several times with chloroform. The chloroform layers were combined, dried over anhydrous sodium sulfate, and then evaporated *in vacuo* to give 181 g of a dark brown gum.

ISOLATION OF ISOHELENALIN (**1**) AND ISOHELENOL (**2**).—The crude chloroform extract was chromatographed on silica gel (10 x 70 cm) and eluted with benzene-chloroform, chloroform, chloroform-ethyl acetate, and acetone. Isohelenalin (**1**, 130 mg) was isolated from the chloroform-ethyl acetate (9:1) fractions. The acetone fractions were combined and rechromatographed on silica gel in chloroform, chloroform-ethyl acetate (20:1), and chloroform-methanol (50:1) to give 0.6 g of crude isohelenol. This gum was purified further by ptlc (chloroform-methanol, 8:1) to give 100 mg of crystalline isohelenol (**2**).

ISOHELENALIN (**1**).—Isohelenalin had mp 222–224° (chloroform-benzene) [Lit. 6 reported mp 260–262°; Lit. 7 reported mp 268–270° (acetone-ether)]. The ir spectrum ($CHCl_3$) of **1** showed bands at 3300–3600 (OH), 1740 (lactone), 1700 and 1575 (cyclopentenone) cm^{-1}. Its ^{1}H-nmr ($CDCl_3$) spectrum exhibited signals at δ 7.72 (1H, dd, J=2.0, 6.0 Hz, H-2), 6.20 (1H, dd, J=3.0, 6.0 Hz, H-3), 5.30 (1H, m, H-8), 5.08 (1H, br. s, H-6), 3.28 (1H, m, H-1), 1.96 (3H, d, J=2.0 Hz, CH_3-11), 1.36 (3H, d, J=6.0 Hz, CH_3-10), and 1.02 (3H, s, CH_3-5). The mass spectrum showed M+ at m/e 262.

ISOHELENOL (**2**).—Isohelenol had mp 190–192°. Its ir ($CHCl_3$) showed bands at 3400 (OH), 1750 (lactone), 1705 and 1580 (cyclopentenone) cm^{-1}. The ^{1}H-nmr ($CDCl_3$) exhibited signals at δ 7.74 (1H, dd, J=2.0, 6.0 Hz, H-2), 6.20 (1H, dd, J=3.0, 6.0 Hz), 5.38 (1H, m, H-8), 5.18 (1H, br. s, H-6), 4.46 (2H, br. s, CH_2-11), 3.45 (1H, m, H-1), 1.36 (3H, d, J=6.0 Hz, CH_3-10) and 1.02 (3H, s, CH_3-5). Its ^{13}C-nmr spectrum (pyridine) exhibited peaks at δ 210.97 (s, C-4), 173.14 (s, C-12), 166.80 (s, C-7), 165.03 (d, C-2), 131.31 (d, C-3), 129.26 (s, C-11), 77.74 (d, C-8), 65.93 (d, C-6), 55.41 (s, C-5), 53.88 (t, C-13), 49.54 (d, C-1), 39.78 (t, C-9), 26.68 (d, C-10), 21.22 (q, C-14) and 18.75 (q, C-15). The mass spectrum showed M+ at m/e 278.1157 ($C_{15}H_{18}O_5$ requires 278.1153).

ACETYLATION OF ISOHELENOL.—Acetylation of isohelenol (**2**, 10 mg) with acetic anhydride (0.5 ml) and pyridine (1 ml) at room temperature for 18 hours followed by the usual work-up and ptlc (chloroform-acetone, 5:1) gave 5 mg of diacetyl isohelenol (**3**). The mass spectrum of **3** showed M+ at m/e 362.1368 ($C_{19}H_{22}O_7$ requires 362.1364). Its ^{1}H-nmr ($CDCl_3$) exhibited peaks at δ 7.70 (1H, dd, J=2.0, 6.0 Hz, H-2), 6.24 (1H, dd, J=3.0, 6.0 Hz, H-3), 6.21 (1H, br. s, H-6), 5.15 (1H, m, H-8), 5.05 (2H, AB q, J=13.0 Hz, 11-CH_2), 3.1 (1H, m, H-1), 2.10 and 1.96 (both 3H, s, $OCOCH_3$), 1.38 (3H, d, J=6.0 Hz, CH_3-10) and 1.10 (3H, s, CH_3-5).

OXIDATION OF ISOHELENALIN.—Pyridinium chlorochromate (50 mg) was added with stirring to isohelenalin (**1**, 10 mg) in 10 ml of ice-cold anhydrous methylene chloride. After 10 minutes the solution was allowed to warm to room temperature and stirred overnight. The solution was then diluted with chloroform, washed with water, dried over anhydrous sodium sulfate, and the solvent evaporated *in vacuo*. The residual oil was chromatographed on silica gel column (chloroform-ethyl acetate, 9:1), followed by ptlc (chloroform-ethyl acetate, 1:1) to give a compound which was recrystallized from chloroform-ether to yield 6 mg of **6**: mp 162–163°; ir ($CHCl_3$): 1750, 1715 and 1680 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 7.56 (1H, dd, J=2.0, 6.0 Hz, H-2), 6.16 (1H, dd, J=3.0, 6.0 Hz, H-3), 5.22 (1H, m, H-8), 3.00 (1H, br. d, J=12 Hz, H-1), 2.15 (3-H, br. s, CH_3-11), 1.48 (3H, s, CH_3-5) and 1.44 (3H, d, J=6.0 Hz, CH_3-10). The mass spectrum of **6** showed M+ at m/e 260.

OXIDATION OF HELENALIN.—Pyridinium chlorochromate (100 mg) was added with stirring to 50 mg of helenalin (**4**) in 50 ml of ice-cold anhydrous methylene chloride. After 10 minutes the solution was allowed to warm to room temperature and stirred overnight. The reaction was worked up as above. The oil was purified as above to give 13 mg of a crystalline product (**6**, mp 161–163°) which was identical by tlc and ir, nmr and mass spectra to that obtained from the oxidation of isohelenalin (**1**).

REDUCTION OF ISOHELENALIN.—Platinum oxide (3 mg) was added to 9 mg of isohelenalin (**1**) in 10 ml of ethanol. The solution was stirred under hydrogen gas for 6 hours, the catalyst filtered out and the solvent removed. The residue was recrystallized from chloroform-ether to give 8 mg of **7** as colorless needles: mp 206–208° (Lit. 6 reported mp 212°). Its ir spectrum ($CHCl_3$) showed hydroxyl (3400 cm^{-1}) and carbonyl (1730 cm^{-1}) peaks. The ^{1}H-nmr ($CDCl_3$) showed signals at δ 5.15 (1H, m, H-8), 4.88 (1H, br. s, H-6), 1.89 (3H, br. s, CH_3-11), 1.19 (3H, d, J=6.0 Hz, CH_3-10) and 0.84 (3H, s, CH_3-5). The mass spectrum showed M+ at m/e 264.

ACKNOWLEDGMENTS

This investigation was supported by grants from the National Cancer Institute (CA 17625) and the American Cancer Society (CH-19) to K. H. Lee. The authors thank Professor Iris H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina

at Chapel Hill, for biological assay; Dr. David L. Harris, Department of Chemistry, University of North Carolina at Chapel Hill for nmr spectra, and Dr. David Rosenthal and Mr. Fred Williams, Research Triangle Center for Mass Spectrometry, for mass spectral data.

Received 26 December 1978.

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