Antitumor Agents XVII: Structure and Stereochemistry of Microhelenin-A, a New Antitumor Sesquiterpene Lactone from *Helenium microcephalum*

Keyphrases □ Antitumor agents, potential—microhelenin-A, isolated from *Helenium microcephalum*, NMR spectral identification □ Microhelenin-A—potential antitumor agent, isolated from *Helenium microcephalum*, NMR spectral identification □ *Helenium microcephalum*—microhelenin-A, potential antitumor agent, isolated and identified

To the Editor:

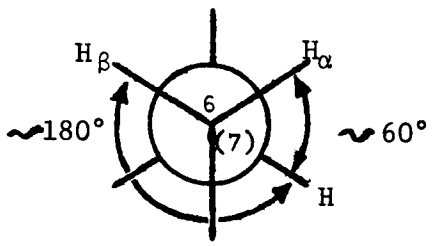
The search for a supply of helenalin for investigations on the relationship between the sesquiterpene lactone structure and cytotoxic antitumor activity (1) led to the isolation of a new antitumor[1] (2) sesquiterpene lactone, microhelenin-A (Structure I), from *Helenium microcephalum*[2] (3).

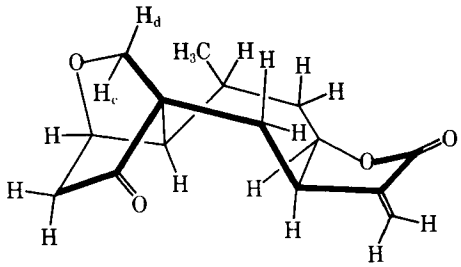
Microhelenin-A was isolated as colorless needles in 0.005% yield from the chloroform extract of *H. microcephalum* according to an exact literature procedure (4) followed by silica gel column chromatography. Microhelenin-A [mp 140–141°, $[\alpha]_D^{24}$ +89° (c, 1 in methanol[3], *m/e* 262 (M^+)] has the molecular formula[4] $C_{15}H_{18}O_4$ and showed the presence of a cyclopentanone ring system (1758 cm^{-1}). The presence of an α-methylene-γ-lactone moiety in Structure I was indicated by the appearance of IR bands (CCl_4) at 1772 and 1665 cm^{-1} and was substantiated by the presence in the NMR spectrum[5] of a characteristic pair of low field doublets at δ 5.77 (1H, J = 3.0 Hz, H_a − 13) and 6.34 (1H, J = 3.0 Hz, H_b − 13).

A three-proton doublet at δ 1.17 (3H, J = 7.0 Hz) was ascribed to the secondary methyl group at C-10. The one-proton signals at δ 4.77 (ddd, J = 4.0, 9.0, and 11.0 Hz) and 3.12 (m) were assigned to the hydrogens at C-8 and C-7, respectively, since irradiation at C-7 caused the signal at C-8 to collapse to a doublet of doublets at δ 4.80 (J = 4.0 and 11.0 Hz) and caused the doublet signals for H_a − 13 and H_b − 13 to collapse to two singlets at δ 5.77 and 6.34, respectively. This evidence led to the assignment of the partial Structure II to microhelenin-A.

Microhelenin-A (Structure II) and 2,3-dihydrohelenalin (III) (6) both exhibited the comparable negative Cotton effects in circular dichroism[6] (CD) studies (Structure II: $[\theta]_{253}$ −3144°; III: $[\theta]_{262}$ −3748°) and positive Cotton effects of the optical rotatory dispersion[6] (ORD) curves (Structure II: $[\Phi]_{313}$ +5633°; III: $[\Phi]_{315}$ +5016°), indicating that both possess the same C-7/C-8 *cis*-fused lactones with H-7α and H-8α as well as the *trans*-fused *A/B* rings with H-1α and alkyl-5β (7).

The NMR spectrum of I also displayed an *AB* quartet (J = 9.0 Hz)[7] at δ 3.64 (1H) and 3.85 (1H) as well as a multiplet at δ 4.53 (1H), which were assigned to the protons of —CH_2OCH—. Determination of the conformation of the B-ring as well as the position of the —CH_2OCH— linkage was achieved by an INDOR experiment (8–10). As illustrated in Fig. 1, when the quartet lines of H-6β (ax) (δ 1.67, dd, J = 13.0 and 15.0 Hz) were monitored, they gave the INDOR signals for H-6α (eq) around δ 2.30–2.50, indicating that the central seven-membered ring of Structure I (see also Structure I*a*) adopts a boat conformation:





I*a*

with CH_2-5β axially disposed. The observed J values between H-6β and H-7α (J = 13.0 Hz) and between H-6α and H-7α (J = 3.0 Hz) are in accord with those derived from the Karplus rule (11), since models indicate that the dihedral angle between H-6β and H-7α is approximately 180° and that between H-6α and H-7α is approximately 60°. The downfield shift of H-6α in comparison to H-6β to δ 2.44 (dd, J = 3.0 and 15.0 Hz) is due to the coplanarity of H-6α with the carbonyl at C-4.

The position for the —CH_2OC*H*— linkage was determined as follows. The NMR data indicated the presence of a secondary methyl group at C-10 and a methylene proton at C-9. Consequently, the methine

[1] Microhelenin-A showed significant (T/C ≥125%) inhibitory activity against the Walker 256 carcinosarcoma in rats (T/C 148%) at the 2.5-mg/kg level. *In vivo* activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, by a literature method (2).

[2] Specimens were gathered in June 1972 in Burleson County, Tex. We thank Professor John J. Sperry, Texas A&M University, for collecting and identifying the plant material. 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. The constituents of *H. microcephalum* were previously examined and reported to contain helenalin in good yield (3).

[3] Measurements were carried out on a Perkin-Elmer model 141 polarimeter. Mexicanin-H showed $[\alpha]_D$ −44.6° (see Ref. 5).

[4] Determined by high-resolution mass spectrometry.

[5] NMR spectra were measured in deuterochloroform (tetramethylsilane) with a Varian XL-100-FT instrument.

[6] CD and ORD curves were measured in methanol (c, 0.1%) on a Cary 60 recording spectropolarimeter.

[7] This quartet became a singlet at δ 3.38 when Structure I was measured in deuterated benzene–deuterochloroform (1:1).

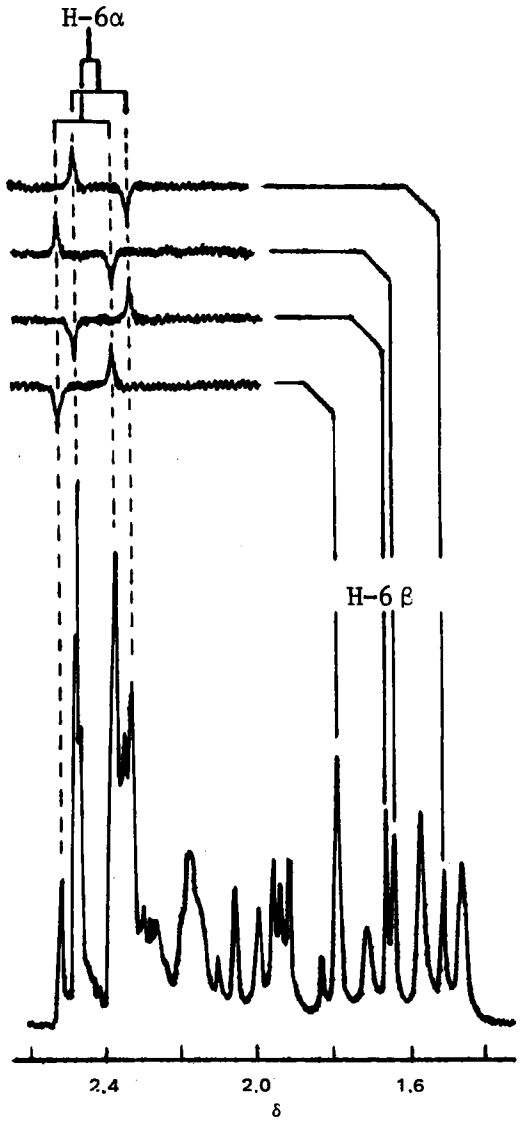
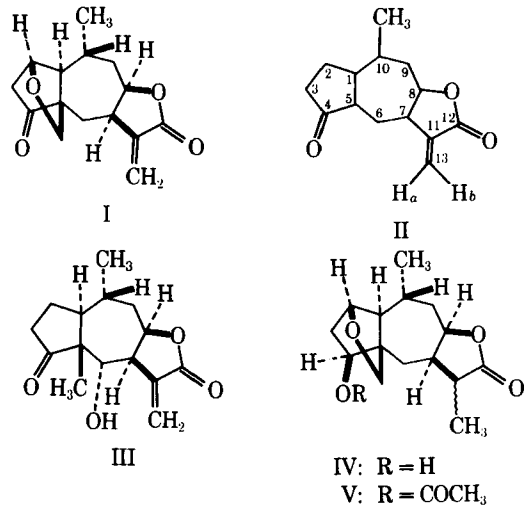


Figure 1—*The 100-MHz normal and INDOR spectra of microhelenin-A.*

proton in —CH_2OCH— can only be placed at either C-2 or C-3. Of these, the former is regarded as the most likely because monitoring the peak around δ 4.53 (H-2α) gave INDOR signals arising from H-1α and H-3 at δ 1.90–2.40. The H-1α INDOR signal would not be observed if the linkage is at C-3. Further confirmation for this assignment was obtained by sodium borohydride reduction of Structure I, which afforded a monohydroxydihydro derivative (IV).

Compound IV [$C_{15}H_{22}O_4$; ν_{max} 3590 (OH) and 1762 (γ-lactone) cm^{-1}] showed two three-proton doublets at δ 1.07 (J = 7.0 Hz, CH_3-10) and 1.18 (J = 7.5 Hz, CH_3-11) and a one-proton multiplet at δ 4.78 (H-8). The two-proton multiplet at δ 4.00 was assigned to protons at C-2 and C-4. The downfield shift of H_c, which appeared as a one-proton doublet at δ 4.18 (J = 8.0 Hz), demonstrated that H_c was adjacent to a β-OH group at C-4. The one-proton doublet of doublets (J = 2.0 and 8.0 Hz) at δ 3.50 was assigned to H_d, which was long range coupled to H-4α through an "M" arrangement of the four σ-bonds. Acetylation of IV with acetic anhydride–pyridine yielded a monoacetate [V; δ 2.14 (3H, s, $OCOCH_3$-4), 4.09 (1H, m, H-2), and 4.70–5.10 (2H, m, H-4 and H-8)].



The methyl group at C-10 was assigned to an α-equatorial orientation since it appeared at δ 1.17. One would expect that, if the C-10 methyl was in the β-axial configuration, it would be shifted to a lower field due to the deshielding effect of the adjacent oxygen at C-2. This suggestion was confirmed by the fact that no NOE was observed between this C-10 methyl group and the 5 β-axial proton (H_d).

This evidence led to the assignment of Structure I for microhelenin-A[8,9] (5) beyond doubt.

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[8] "Microhelenin-A" is used to differentiate "mexicanin-H" (5) since both compounds showed different melting point and $[\alpha]_D$ values as well as NMR and mass spectra, although they possess the same plane structure.

[9] This assignment was also confirmed by a single crystal X-ray analysis by Dr. A. T. McPhail and Dr. K. D. Onan of Duke University after the submission of this manuscript.

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Method for Monitoring Hard Gelatin Capsule Disintegration Times in Humans Using External Scintigraphy

Keyphrases □ Gelatin capsules, hard—disintegration and drug release times *in vivo* monitored by external scintigraphy using technetium Tc 99m □ Disintegration time—hard gelatin capsules *in vivo*, monitored by external scintigraphy □ Drug release time—hard gelatin capsules *in vivo*, monitored by external scintigraphy □ Technetium Tc 99m—used in scintigraphic monitoring of disintegration and drug release times of gelatin capsules *in vivo* □ Scintigraphy, external—disintegration and drug release times of gelatin capsules monitored

To the Editor:

We wish to report a noninvasive and novel approach for monitoring disintegration and drug release times from a gelatin capsule in humans. The method involves the utilization of a short-lived radionuclide coupled with external scintigraphy.

Two separate formulations were used to fill identical hard gelatin capsules[1]. Formulation A consisted of 150 mg of a totally water-insoluble polystyrene resin (40–100 mesh) bearing polyamine functions which chelated the radionuclide, technetium Tc 99m, in an irreversible manner (1). Formulation B was water soluble, consisting of 145 mg of lactose and 5.9 mg of the soluble chelating agent etidronate disodium[2] labeled with technetium Tc 99m.

In a typical experiment, the subject ingested a gelatin capsule[1] filled with an appropriate material labeled with 20 mCi of technetium Tc 99m. Technetium Tc 99m is a γ-ray-emitting radionuclide (half-life = 6 hr) with an energy of 140 kev. The low energy and short half-life of this radionuclide make it suitable for external scintigraphic studies involving humans, and the radiation dose is minimal.

Following oral administration with 100 ml of water, the normal subject was placed in a supine position on a hard top stretcher for general body immobilization. The abdomen was then positioned beneath the collimated detector of a multicrystal scintillation camera[3]. Data were accumulated for up to 200 min, when necessary, at 1-min intervals. These 1-min count integrations were stored on the computer magnetic tape for future retrieval.

During data collection, scintiphotos were taken. These scintiphotos (Fig. 1) showed the release of radioactivity from the capsule region to the other portions of the stomach as a function of time. The pictures also illustrated the lack of capsule movement within the stomach, the swelling of the capsule, and, finally, the release and dispersion of the capsule contents to other stomach regions (Fig. 1).

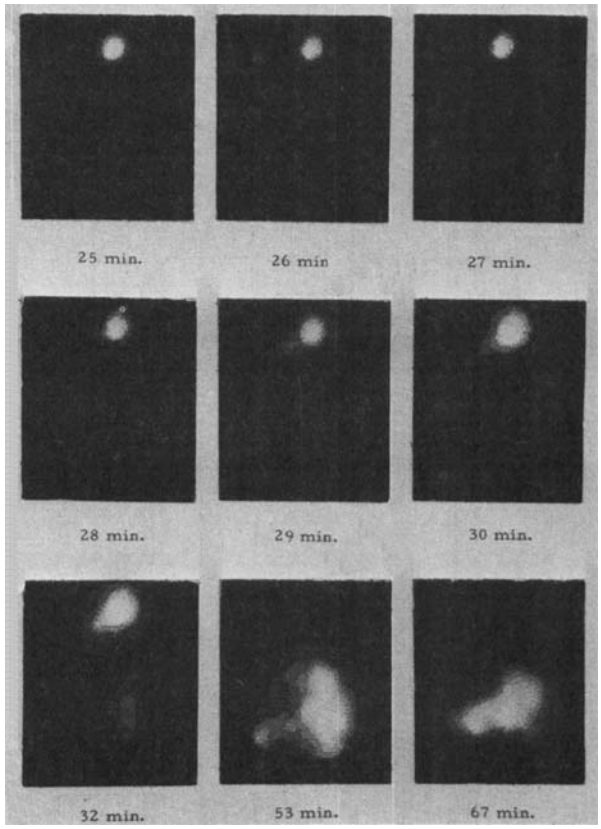

Figure 1—*Sequential scintiphotos of intermittent phases of the* in vivo *collapse of a gelatin capsule using Formulation A (empty stomach).*

Although the scintiphotos gave a qualitative picture of the release of the capsule contents, quantitative determination of the absolute capsule disintegration rate was performed with the aid of a computer. We chose an area of interest directly over the capsule and an area of

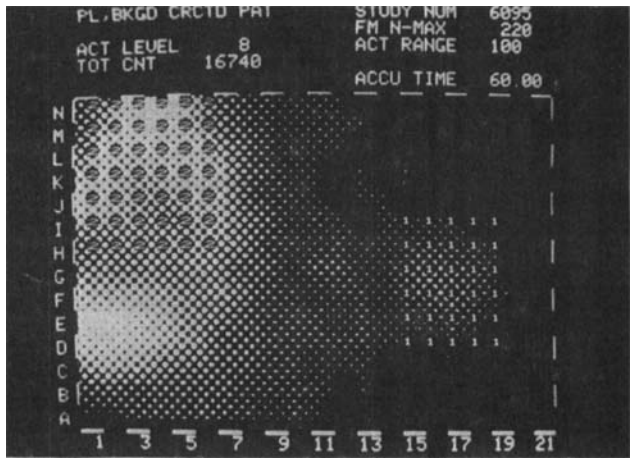

Figure 2—*Scintiphotos showing the two computer areas of interest within the stomach. Key: 1, area of interest over the capsule; and 2, area of interest over the pyloric region of the stomach.*

[1] Gelatin capsules No. 0, Eli Lilly and Co., Indianapolis, IN 46206
[2] Osteoscan, Procter and Gamble Co., Cincinnati, Ohio.
[3] Gamma-camera (Baird Atomic-System 77) equipped with a computer and magnetic tape and possessing storage and replay capability.