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ACKNOWLEDGMENTS AND ADDRESSES

Received January 17, 1974, from the *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication March 27, 1974.

The authors thank Mr. W. L. Wilham for technical assistance and Mr. L. L. Simms for help in computerization of the mathematical treatments.

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Tumor-Inhibitory Agent from *Zaluzania robinsonii* (Compositae)

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Abstract □ The chloroform extract of *Zaluzania robinsonii* Sharp has shown activity against the P-388 lymphocytic leukemia test system. The constituent responsible for this activity was a guaianolide, identified as zaluzanin C ($C_{15}H_{18}O_3$). The identity was proven by melting point, mixed melting point, elemental analysis, IR, PMR, mass spectroscopy, and preparation of derivatives.

Keyphrases □ *Zaluzania robinsonii* (Compositae)—isolation and identification of zaluzanin C as tumor-inhibiting agent □ Zaluzanin C—isolation and identification from *Z. robinsonii*, tested for tumor-inhibitory activity □ Antitumor agents, potential—isolation, identification, and screening of zaluzanin C from *Z. robinsonii*

As a result of the continuing search for plants having tumor-inhibiting constituents, the ethanol extract of the leaves, stems, flowers, and roots of *Zaluzania robinsonii* Sharp (Compositae)[1] was found to have inhibitory activity toward the P-388 lymphocytic leukemia test system (3PS)[2].

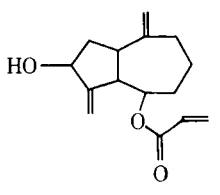
DISCUSSION

The chloroform extract, obtained from an ethanol extract by partition between chloroform and water, was subjected to systematic fractionation employing partition and solvent extraction followed by column chromatography on silica gel. The sequence of steps in the fractionation procedure leading to the isolation of the guaianolide is outlined in Scheme I. The guaianolide was identified as zaluzanin C (I) by means of its melting point, elemental analysis, mass spectroscopy, and comparison of IR and PMR spectra with authentic sample spectra. In addition, no depression in a mixed melting point and a superimposable IR spectrum of the acetyl derivative with an authentic sample and identical physical data of the dehydro derivative further proved the identification of the guaianolide as zaluzanin C. The compound had been previously isolated from two closely related species, *Z. augusta* (lag) Schultz, Bip. (1) and *Z. triloba* Pers. (2).

This compound demonstrated an activity of 161% test/control (T/C) at 150 mg/kg in the 3PS test system. Activity in the 3PS system is defined as an increase in the survival of treated animals over that of controls resulting in a T/C ≥ 125% (3).

EXPERIMENTAL[3]

Isolation Procedure—The roots, stems, leaves, and flowers (8.5 kg) of *Z. robinsonii* were ground and extracted exhaustively in a Lloyd-type extractor with ethanol. After removal of the solvent in air, the residue was subjected to three-funnel partition in three lots between benzene–methanol–water (8:5:1) using 1700 ml of each phase. All upper phases were combined, the solvent was removed in air, and the residue was stirred mechanically twice with ethyl acetate and filtered. The combined ethyl acetate-soluble fraction yielded a residue weighing 46 g. The lower phases (second and third) obtained from these partitions were similarly combined and the solvent was removed. The residue was stirred twice with *n*-hexane and filtered. The hexane-insoluble residue, weighing 8.5 g, was then mixed with the ethyl acetate-soluble residue (46 g). A portion of this mixture (39 g) was subjected to silica gel (1700 g) column (10 × 152 cm) chromatography. The column was eluted



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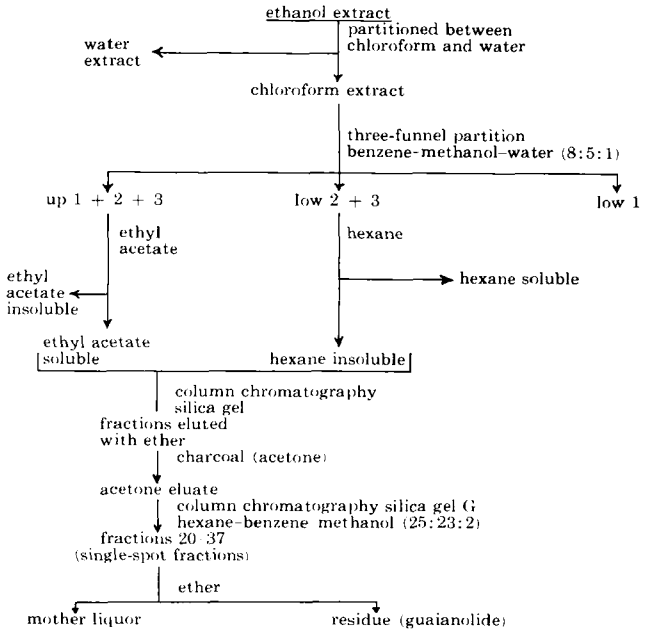
[1] Identification was confirmed by Dr. Robert E. Perdue, Medicinal Plant Resources Laboratory, Plant Genetics and Germ Plasm Institute, Beltsville, Md. A reference specimen was deposited in that herbarium. The plant was collected in San Luis Potosi, Mexico, in August 1970.

[2] Of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md.

[3] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. The melting points were determined on a Kofler hot-stage apparatus and are uncorrected.



Scheme I—Isolation of zaluzanin C

with various solvents, starting with hexane, with increasing order of polarity. The fraction (20 g) from the ether eluate, which showed essentially one large spot on TLC, demonstrated antitumor activity.

Isolation of Zaluzanin C—A portion of this active fraction (6 g) was adsorbed on activated charcoal, and the resulting dry pulverized adsorbate was placed on the top of a column packed with a small amount of sand and eluted with acetone. The residue (5.07 g) obtained after removal of the solvent *in vacuo* was subjected to silica gel (60 g) column (3 × 68 cm) chromatography using hexane–benzene–methanol (25:23:2) as the solvent system for elution. Fractions (20–37; each fraction 15 ml) displaying a single spot on TLC were combined and the solvent was removed *in vacuo*. The resulting residue, on treatment with ether (15 ml), gave nearly colorless crystals of zaluzanin C. The mixture was filtered after standing overnight in the refrigerator and was washed with a small amount of cold ether to give 1.09 g, mp 93–94°. Crystallization from acetone–hexane afforded pure zaluzanin C in colorless prisms, mp 94–95° [lit. (2) mp 95–96°], $[\alpha]_D^{27}$ +38° (c 1% in $CHCl_3$). The IR and PMR spectra were identical with the authentic sample spectra[4]. Mass spectrometry indicated a parent peak of 246.

Anal.—Calc. for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37; mol. wt. 246. Found: C, 73.06; H, 7.58; *m/e* 246.

Zaluzanin C Acetate—Acetylation of zaluzanin C with acetic anhydride in pyridine for 2 hr at room temperature yielded the acetate, which crystallized from hexane–isopropyl ether as colorless plates, mp 100°, $[\alpha]_D^{27}$ +24°(c 1% in $CHCl_3$). The mixed melting point with the authentic sample[4] showed no depression. The IR spectra were superimposable. Mass spectrometry indicated a parent peak of 288.

Dehydrozaluzanin C—Chromic acid oxidation of zaluzanin C in pyridine at ice-cold temperature yielded the dehydro product, which was purified by preparative TLC followed by crystallization from acetone–hexane, mp 132–133°; IR maxima (KBr): 1760, 1720, 1632, and 885 cm^{-1} (3).

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 6, 1974, from the *Division of Pharmaceutical Chemistry, College of Pharmacy, University of Arizona, Tucson, AZ 85721*

Accepted for publication April 9, 1974.

Supported in part by Contract NO1-CM-3-3750 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014, and the Elsa U. Pardee Foundation, Midland, Mich.

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[4] The authors are grateful to Dr. J. Romo, Instituto de Quimica, Mexico 20, D.F., for providing the spectra and the sample.