Tumor Inhibitors XII. Gaillardin, A New Cytotoxic Sesquiterpene Lactone from *Gaillardia pulchella*

Sir:

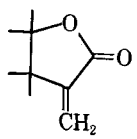
In the course of a continuing search for tumor inhibitors from plant sources, an alcoholic extract of *Gaillardia pulchella* Foug. (*Compositae*)[1] was found to have reproducible inhibitory activity against human carcinoma of the nasopharynx carried in cell culture (KB).[2]

This communication reports the fractionation of the active extract and the isolation and preliminary characterization of a new cytotoxic sesquiterpene lactone, which we have named gaillardin.

Solvent partition of the concentrated alcoholic extract (A in Fig. 1) between chloroform and water resulted in concentration of the activity (Table I) in the chloroform phase (D). Partition of this residue between 10% aqueous methanol and petroleum ether (Skellysolve B) concentrated the activity in the aqueous methanol layer (G). The material recovered from the aqueous methanol layer was redissolved in methanol and treated with excess saturated methanolic neutral lead acetate solution. Removal of the precipitate by filtration and of the excess lead with hydrogen sulfide yielded a still more active fraction (I).

Fraction I was chromatographed on a silicic acid–Celite (3:1) column, and fraction L, eluted with chloroform, was found to be enriched in a compound which gave a violet color reaction with antimony trichloride spray reagent (1) upon thin-layer chromatography.[3] Rechromatography twice by the same procedure yielded a material of R_f 0.58, which was crystallized thrice from benzene–petroleum ether to yield gaillardin, in the form of colorless needles, m.p. 198–199°; $[\alpha]_D^{30}$ −15° (c 1.08, chf.); $\lambda_{max.}^{EtOH}$ end absorption at 209 mμ (ε 15,500); $\lambda_{max.}^{chf.}$ 2.78, 5.67, 5.78, 6.00, 6.04, 8.15 μ; NMR signals at 3.79 τ, 1H, d, J=3 c.p.s.; 4.47 τ, 1H, d, J=3 c.p.s.; 7.92τ, 3H, s; 8.18 τ, 3H, broad s; 8.74τ, 3H, s.[4]

The molecular formula, $C_{17}H_{22}O_5$, was assigned for gaillardin on the basis of elemental analysis and molecular weight determination (m/e 306) by mass spectrometry.[5] Assignment to the sesquiterpene lactone group is proposed on the basis of the molecular formula and spectral properties of gaillardin. Thus, the presence in the infrared spectrum of adsorption at 5.67 μ (α,β-unsaturated γ-lactone) and 6.04 μ (conjugated double bond) indicates the presence of the characteristic γ-lactone containing a conjugated exocyclic methylene group (partial structure I). This partial structure is supported by the presence in the NMR spectrum of two low-field doublets (3.79τ, 1H, and 4.47τ, 1H, J=3 c.p.s.) which are characteristic for the exocyclic methylene (2, 3). The presence of an acetate grouping in gaillardin is indicated by the infrared absorption at 5.78 and 8.15 μ, a sharp singlet at 7.92τ in the NMR spectrum, and a characteristic M-60 peak at m/e = 246 in the mass spectrum of gaillardin.[5] The infrared absorption at 2.78 μ



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shows the presence of a hydroxyl group, accounting for the fifth oxygen atom of gaillardin. The infrared absorption at 6.00 μ suggests that gaillardin possesses a second double bond. Further studies aimed at the elucidation of the structure of gaillardin are in progress.[6]

The cytotoxicity of an unsaturated lactone of the sesquiterpene group is noteworthy, particularly in view of earlier observations concerning the growth inhibitory activity of monocyclic unsaturated lactones (4) and of cardenolide derivatives (5, 6). Structural studies of other

[1] Roots, stems, leaves, and flowers were gathered in Texas, May 1964. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program development with the USDA by the Cancer Chemotherapy National Service Center.

[2] Cytotoxicity was assayed, under the auspices of the CCNSC, against Eagle's KB strain of human epidermoid carcinoma. The procedures were those described in *Cancer Chemotherapy Repl.*, **25**, 1(1962).

[3] Thin-layer chromatography was conducted on Merck Silica Gel G using 10% methanol in chloroform as solvent.

[4] The NMR spectrum was determined in deuterochloroform solution on a Varian A-60 spectrometer with TMS as internal standard.

[5] The authors thank Professor A. L. Burlingame, University of California, Berkeley, for the mass spectral data.

[6] Herz and co-workers have recently reported isolation and structural studies on four other sesquiterpene lactones from *G. pulchella* Foug. (2, 3).

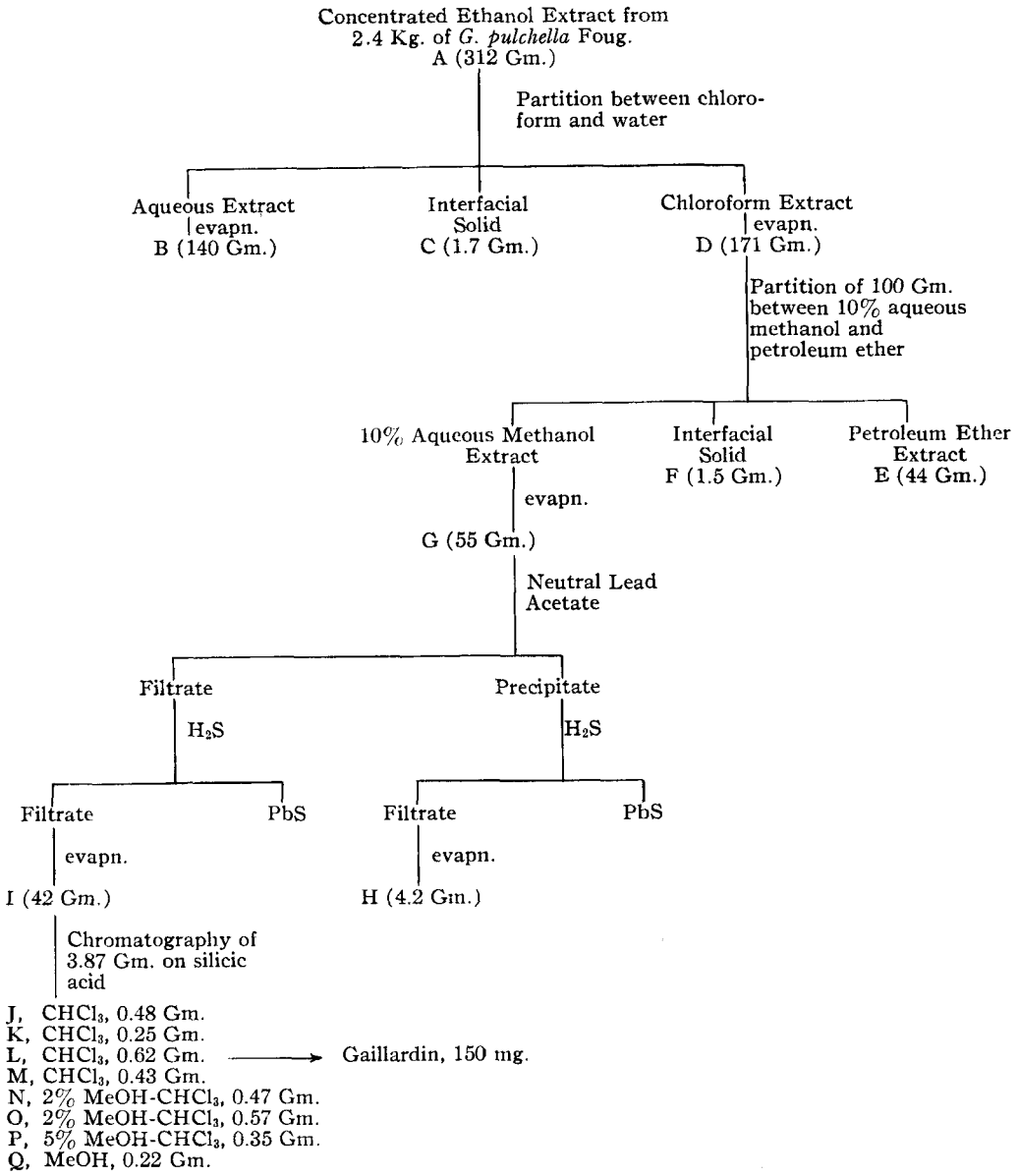

Fig. 1.—Flow sheet for fractionation of cytotoxic extract from *G. pulchella* Foug.

TABLE I.—CYTOTOXICITY OF FRACTIONS FROM *G. pulchella* FOUG.

Fraction	ED_{50}, mcg./ml.	Fraction	ED_{50}, mcg./ml.
A	1.9	J	0.52
B	100	K	14
C	46	L	5.2
D	4.0	M	1.7
E	53	N	3.2
F	23	O	2.0
G	2.0	P	5.0
H	25	Q	1.3
I	0.27	Gaillardin	0.80, 1.6, 2.3[a]

[a] The biological assay data indicate that gaillardin is responsible for only a small fraction of the cytotoxicity of the extract of *G. pulchella*. Studies of other cytotoxic constituents are in progress.

cytotoxic unsaturated lactones from *G. pulchella* Foug. and other plants are in progress and will be reported in due course.

(1) Cekan, Z., Herout, V., and Sorm, F., *Collection Czech. Chem. Commun.*, **22**, 1921(1957).
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(6) Kupchan, S. M., *et al.*, *Science*, **146**, 1685(1964).

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Added in proof: The authors thank Professor W. Herz for informing us recently of his observation that other sesquiterpenes containing the α,β-unsaturated lactone system I were also active in the CCNSC KB cell culture cytotoxicity assay.

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Books

REVIEWS

Chemical-Biological Activities. March 22, 1965. Vol. 1, No. 6. A publication of the Chemical Abstracts Service. Published by the American Chemical Society, 20th and Northampton Sts., Easton, Pa., 1965. 8.5 × 11 cm. Paperbound. Price: Subscription rates 1965, $750. per year plus $5 for each scientist; single issues, $35.

Chemical-Biological Activities (*CBAC*) is a new and specialized service of Chemical Abstracts Service (CAS). *CBAC* is a computer-based index to the current literature of biological activities of organic compounds. Compounds which have an effect on animals, but not plants, are included. The computer processing permits rapid dissemination of research results; an article may be noted within 3 weeks of its original publication.

CBAC does not abstract entire articles, but presents in concise summary sentences the results of the study. The summaries follow a standard form because of the computer technique used and each contains the compound(s) studied, the effect produced, the biological system affected, and the animal used. Each item is accompanied with a CAS registry number for computer retrieval purposes. The computer store should be large enough to be useful in searching by January 1966. Each digest entry contains a complete journal reference. Structures are included if these are not currently listed in either the "Merck Index" or "United States Adopted Names."

Three indexes are included, keyword-in-context (KWIC), molecular formula, and author. The latter two are straightforward and easy to use. The KWIC index contains fragments of the title and digest entry rearranged and alphabetized according to various *key* words in the entry. At first glance it appears confusing, and its practical value can be determined only after some usage. Indexed issues are published every other week, covering journals 1 to 9 months old. Cumulative indexes are prepared every 6 months. Three hundred of the most important chemical, biological, medical, and pharmaceutical journals are annotated. The *Journal of Pharmaceutical Sciences* is included.

Isotopes in Experimental Pharmacology. Edited by Lloyd J. Roth. The University of Chicago Press, 5750 Ellis Ave., Chicago, Ill., 1965. xiv + 488 pp. 15 × 23.5 cm. Price $12.50.

This book, admirably well edited by Dr. Lloyd J. Roth, Professor and Chairman of the Department of Pharmacology, The University of Chicago, is a compendium of lectures from an International Conference on the Uses of Isotopically Labeled Drugs in Experimental Pharmacology held in Chicago, June 7 to 9, 1964.

Fulfilling the prophecy of Schoenheimer and Rittenberg in 1935 regarding the almost unlimited applications of isotopes in the study of intermediary metabolism, Dr. Roth then presents the work of 40 outstanding international authorities in their respective applications of isotopes to pharmacology. The studies are presented in Parts I to VII which are entitled: Isotopic Labeling of Drugs Activation Analysis, Autoradiography, Compartmental Analysis and Dynamic Measurements, Drug Biotransformation, Biochemical Pharmacology, and Deuterium Isotope Effect, Elucidation of Pharmacological Mechanisms, respectively.

These major divisions are further subdivided into 36 chapters by the contributors who present a variety of disciplines and techniques using isotopes in their multifaceted pharmacological research. These contributions are sufficiently detailed with adequate references so as to supply the researcher or reader with suggested models for similar studies.

The feasibility of the use of labeled drugs to study aspects of pharmacology, such as distribution, metabolism, and retention is amply demonstrated. The employment of the labeled drugs frequently leads to a simplification and positivity of the techniques which would not have been possible otherwise.

This volume is highly recommended to pharmacologists, students, and others involved in the pharmaceutical sciences.

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