rates of decline of pharmacologic activity described in the pharmacologic literature. For example, the time course of mydriatic response by mice to atropine is apparently zero order; this is particularly interesting in view of the excellent linearity of the response *versus* log dose plot obtained in the same study (4). The time course of mydriatic response to two other anticholinergic drugs has been described recently in terms of first-order kinetics (5), yet the data are, in fact, much more representative of zero-order kinetics (for example, see Fig. 1). Naturally, the occurrence of apparent zero-order kinetics in the decline of pharmacologic activity, while consistent with the kinetic relationship developed here, cannot prove its existence. Moreover, none of these comments should be interpreted as ruling out the theoretical basis for the occurrence of first- or second-order kinetics with respect to the rate of decline of some types of pharmacologic activity.

Perhaps the most important conclusion to be derived from the present discussion is this: the frequent reasoning (see, for instance, the references cited by Schaumann and Stoepel (6)) that the pharmacologic effect is likely to decline exponentially because the body drug content does is intrinsically wrong unless the intensity of a pharmacologic effect is a linear function of the dose. The latter occurs quite infrequently, although we have recently reported one such case (7). It seems desirable, therefore, to be circumspect in depicting activity *versus* time data and to consider the possibility of a linear decline of activity with time.

The relationship expressed in Eq. 5 has interesting implications. It indicates that, despite a direct relation between body drug content and intensity of the elicited pharmacologic effect, rates of decline of these two quantities can follow different kinetics and, therefore, not be parallel. Since not only $K$ but also $m$ can vary significantly in a series of similar pharmacologic agents (8), the possibility presents itself, through the design and choice of agents with different $K/m$ ratios, of modifying the relationship between the time course of body drug content and the time-intensity course of a pharmacologic effect. This may be a means of reducing certain side effects associated with a given class of chemotherapeutic agents. The $K/m$ ratio may also be a useful additional parameter for characterizing the properties of certain drugs.

(1) Levy, G., in "Prescription Pharmacy," J. B. Lippincott Co., Philadelphia, Pa., 1963, Chapter 2.
(2) Levy, G., "Proc. Annual Research & Scient. Development Conference," Proprietary Association, Washington, D. C., 1961, p. 32.
(3) Randolph L. K., and Cimminera, J. L., in "Remington's Practice of Pharmacy," 12th ed., Mack Publishing Co., Easton, Pa., 1961, Chapter 9.
(4) Quinton, R. M., *J. Pharm. Pharmacol.*, 15, 239(1963).
(5) Sturtevant, F. M., *Proc. Soc. Exptl. Biol. Med.*, 104, 120(1960).
(6) Schaumann, W., and Stoepel, K., *Arch. Exptl. Pathol. Pharmakol.*, 245, 518(1963).
(7) Levy, G., Calesnick, B., and Wase, A., *J. Nucl. Med.*, in press.
(8) Wiberg, G. S., and Stephenson, N. R., *J. Pharm. Pharmacol.*, 13, 416(1961).

GERHARD LEVY

Biopharmaceutics Laboratory
School of Pharmacy
State University of New York at Buffalo
Buffalo

Received December 9, 1963.
Accepted for publication January 10, 1964.
The author has profited greatly from stimulating discussions of this topic with Dr. Eino Nelson and Dr. John G. Wagner and expresses his appreciation to them.

# Tumor Inhibitors III. Monocrotaline, the Active Principle of *Crotalaria spectabilis*

*Sir:*

In the course of a continuing screening program for tumor inhibitors from plant sources, an alcoholic extract of the fruits of *Crotalaria spectabilis* Roth,[1] was found to have reproducible activity against adenocarcinoma 755 in mice.[2] We report herein the fractionation of the active extract and the isolation and characterization of the active principle, monocrotaline.

Preliminary studies of the alcoholic extract indicated that partition between chloroform and water resulted in concentration of the activity in the water phase. Upon treatment of the aqueous solution with dilute aqueous alkali, the active principle was liberated from its salt form and became extractable into chloroform. The systematic procedure which was subsequently developed for isolation of the active alkaloid started with extraction of dried ground plant material in a Soxhlet extractor with 95% ethanol. After concentration of the extract under water pump pressure, a thick brown resinous solid was obtained. Partition between

[1] Gathered in North Carolina, November, 1962. The authors acknowledge with thanks the receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Dept. of Agriculture, Beltsville, Md., in accordance with the program developed with the U.S.D.A. by the Cancer Chemotherapy National Service Center.

[2] Assays were performed by the Wisconsin Alumni Research Foundation under contract to the Cancer Chemotherapy National Service Center. The procedures were those described in *Cancer Chemotherapy Rept.*, 25, 1(1962).

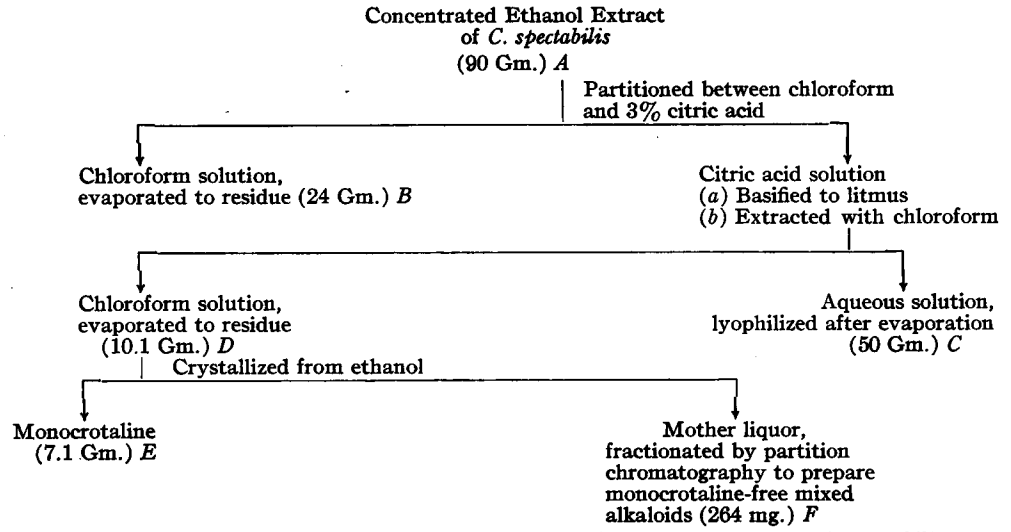



Fig. 1.—Flow sheet for the isolation of the tumor-inhibiting principle from *C. spectabilis*.

TABLE I.—ACTIVITY OF FRACTIONS FROM *C. spectabilis* IN ADENOCARCINOMA 755

| Fraction | Control No. | Dose mg./Kg. | Survivors | Animal Wt. Change Diff. (T-C) | Tumor Wt., mg. (Test/Control) | T/C |
|---|---|---|---|---|---|---|
| *A* | 1536 | 500 | 6/6 | −1.4 | 393/1294 | 30 |
| | | 250 | 5/6 | −1.1 | 798/1294 | 61 |
| | | 125 | 5/6 | −0.4 | 1523/1294 | 117 |
| *B* | 1552 | 300 | 6/6 | −2.7 | 553/974 | 56 |
| | | 150 | 5/6 | −0.2 | 861/974 | 88 |
| | | 75 | 6/6 | −0.6 | 1293/974 | 132 |
| *C* | 1552 | 1000 | 6/6 | −1.1 | 925/974 | 94 |
| | | 500 | 6/6 | −0.8 | 1001/974 | 102 |
| | | 250 | 5/6 | −1.4 | 1228/974 | 126 |
| *D* | 1552 | 200 | 6/6 | −1.9 | 418/974 | 42 |
| | | 100 | 6/6 | −0.9 | 835/974 | 85 |
| | | 50 | 6/6 | −0.1 | 1169/974 | 120 |
| *E* | 1552 | 140 | 6/6 | −1.8 | 274/974 | 28 |
| | | 70 | 6/6 | −1.8 | 352/974 | 36 |
| | | 35 | 6/6 | −0.1 | 600/974 | 61 |
| *F* | 1574 | 100 | 6/6 | −0.2 | 779/907 | 85 |
| | | 50 | 5/6 | −0.5 | 690/907 | 76 |
| | | 25 | 6/6 | +1.0 | 1033/907 | 113 |

chloroform and 3% citric acid solution, followed by basification of the acid solution and extraction with chloroform effected concentration of the active material into a single crude alkaloidal fraction. The latter fraction was readily crystallized from absolute alcohol to afford a high yield of monocrotaline (I).

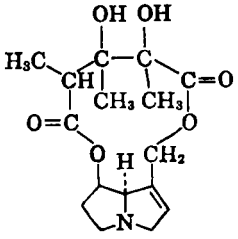
The flow sheet for the isolation is given in Fig. 1; the biological data for the fractions obtained in a typical experiment are reported in Table I. The evaluation of assay results by CCNSC on a statistical basis in sequential testing is such that a material is considered active if it causes reduction of tumor weight to 42% or less.[3] The data in Table I indicate that only subfractions *D* and *E* showed tumor-inhibitory activity.

Paper chromatographic examination of the mother liquors after crystallization of monocrotaline (using Whatman No. 2 paper and a solvent system of 1 part 5% acetic acid equilibrated with 1 part *n*-butanol (1)) revealed the presence of two other alkaloids of lower $R_f$ value. Preparative partition chromatography on Whatman paper powder (standard) with the same solvent system afforded a monocrotaline-free alkaloid mixture (*F*). The absence of significant tumor-inhibitory activity from fraction *F*, coupled with the high yield of monocrotaline isolated from the active concentrate *D*, support the conclusion that monocrotaline was principally —if not solely—responsible for the tumor-inhibitory activity of the alcoholic extract of *C. spectabilis*.

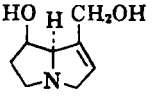
Characterization of the active principle as monocrotaline (I) was effected by comparing the melting points of the alkaloid and its picrate and methiodide derivatives with reported values (2) and the infrared spectrum of the alkaloid with the recorded spectrum (3). In addition, hydrolysis with barium hydroxide solution afforded retrone-

[3] For further details compare protocols described in the reference in *Footnote 2*.

cine (II), characterized by comparing the melting points of the base and its hydrochloride with reported values (4).



I



II

(1) Culvenor, C. C. J., and Smith, L. W., *Australian J. Chem.*, 10, 474(1957).

(2) Adams, R., Shafer, P. R., and Braun, B. D., *J. Am. Chem. Soc.*, 74, 5612(1952).
(3) *Ibid.*, 74, 5878(1952).
(4) For a review of "Senecio Alkaloids," see Leonard, N. J., in Manske, R. H. F., "The Alkaloids," Vol. VI, Academic Press, Inc., New York, N. Y., 1960.

S. Morris Kupchan
Raymond W. Doskotch*
Peter W. Vanevenhoven

Department of Pharmaceutical Chemistry
University of Wisconsin
Madison

Received October 29, 1963.
Accepted for publication November 11, 1963.
This investigation was supported by research grants from the National Institutes of Health (CY-4500) and the American Cancer Society (T-275).
* Present address: College of Pharmacy, Ohio State University, Columbus.

# Books

## REVIEWS

*Organic Syntheses.* Collective Vol. IV. Edited by Norman Rabjohn. John Wiley & Sons, Inc., 605 Third Ave., New York 16, N. Y., 1963. xiv + 1036 pp. 15 × 23 cm. Price $16.50.

This ten-year collective volume covers the materials in the annual volumes 30–39. This book provides the name of the laboratory where the procedure was developed as the first reference for each procedure and also includes an author index. Illustrations of several pieces of equipment or illustrated suggested modifications in equipment are provided. Errors in the original printings of some of the procedures have been corrected, calculations and references checked, and modifications or improvements incorporated. The volume retains the many distinctive features of previous collective volumes which have made them such valuable laboratory tools.

*Progress in Physical Organic Chemistry.* Vol. 1. Edited by S. G. Cohen, A. Streitwieser, Jr., and R. W. Taft. Interscience Publishers, 605 Third Ave., New York 16, N. Y., 1963. ix + 411 pp. 15.5 × 23 cm. Price $15.

Interscience Publishers present the first volume of a new series edited by Saul G. Cohen of Brandeis University, Andrew Streitwieser, Jr., of the University of California (Berkeley), and Robert W. Taft of the Pennsylvania State University covering the relatively modern field of physical organic chemistry. The series is designed to provide a forum for the exchange of views and for critical and authoritative reviews of topics reported in the contemporary outpouring of articles in the field. The authors of the individual sections have been given wide discretion so that each topic discussed should show an individualistic treatment. Treatment of subjects will be more detailed than the conventional textbook style so as to be useful to graduate and practicing organic chemists who may not have any particular expertise in physical organic chemistry. The topics presented in this volume are ionization potentials in organic chemistry, nucleophilic aromatic substitution reactions, ionization and dissociation equilibria in solution in liquid sulfur dioxide, secondary isotope effects, and quantitative comparisons of weak organic bases.

*Papers on Human Genetics.* Edited by S. H. Boyer, IV. Prentice-Hall, Inc., Englewood Cliffs, N. J., 1963. x + 305 pp. 15 × 23 cm. Price $9.

A collection of papers originally published in technical and scientific journals providing background materials on human genetics is presented. One outstanding contribution of this work is providing an English translation of some of the reports which were originally published in German or French. Brief introductory annotations have been included. The collection is designed to illustrate the various ways in which the study of human genetics has proceeded. The examples have been chosen primarily to benefit the newcomers: the students of various rank and training who have become intrigued with human genetics and its problems and wish to have some firsthand knowledge of its origins.

*Identification of Organic Compounds.* By N. D. Cheronis and J. B. Entrikin. Interscience Publishers, 605 Third Ave., New York 16, N. Y., 1963. xii + 477 pp. 15.5 × 24 cm. Price $8.95.

An abridgment and revision of "Semimicro Qualitative Organic Analysis" incorporating materials which are either essential or useful to students of elementary or intermediate organic chemistry is presented. It includes the discussions of, and procedures for, the classification of an unknown substance by its solubility behavior and its acid-base characteristics, general tests for structure and functional groups, specific tests for chemical classes, and an entirely new chapter on the use of paper chromatography and infrared spectroscopy in the identification of organic substances. The volume