equivalent to the activity of 12 mg. of dried digitalis leaf.[2]

Digitalinum verum and verodoxin have been identified tentatively in *D. mertonensis* by thin-layer chromatography (12). *Digitalinum verum* has been shown to have cardiotonic activity in cats (13). Hence, it could be one of the active glycosides. From the data obtained thus far, it also seems that the glycosides present in the callus tissue are more active per gram weight of total glycosides as compared with those present in the leaf.

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Received November 17, 1966.
Accepted for publication February 16, 1967.

[2] U.S.P. digitalis reference standard (6).

Tumor Inhibitors XXII. Senecionine and Senecionine *N*-Oxide, the Active Principles of *Senecio triangularis*

Sir:

In the course of the continuing search for tumor inhibitors from plant sources, an alcoholic extract of *Senecio triangularis* Hook. (*Compositae*)[1] was found to have reproducible activity against the Walker 256 carcinosarcoma tumor (intramuscular) in rats.[2] The fractionation of the active extract and the isolation and characterization of the active principles, senecionine (I) and senecionine *N*-oxide (II), are reported here. Senecionine has been isolated from *Senecio* species and other plants of the *Compositae* and *Leguminosae* (1, 2). While indirect evidence has been advanced for the occurrence of senecionine *N*-oxide in several plants (3–6), the isolation and characterization of the compound have not been reported previously.

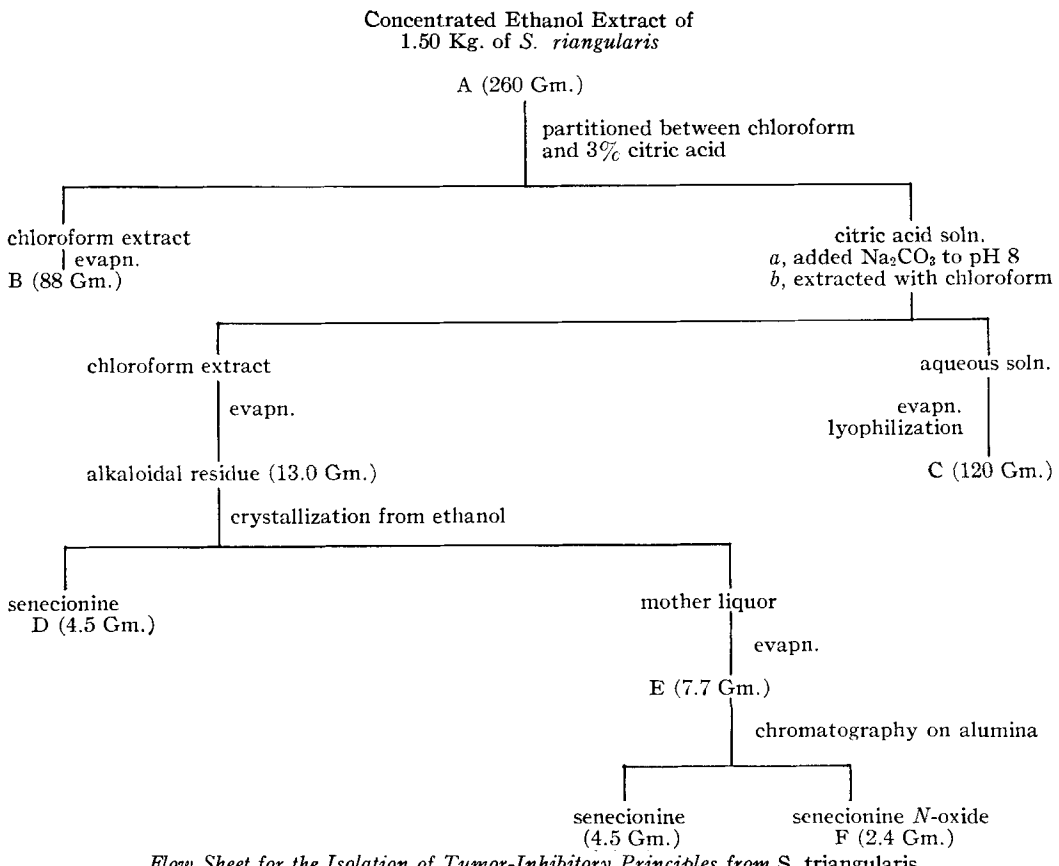
The systematic fractionation of the alcoholic extract of *S. triangularis* is summarized in Scheme I; the *in vivo* assay data for the fractions obtained in a typical experiment are reported in Table I. The evaluation of assay results by CCNSC on a statistical basis in sequential testing is such that a material is considered active if it causes reduction of tumor weight to 42% or less.[2] The absence of tumor inhibitory activity from fractions B and C, coupled with the high yields of senecionine and senecionine *N*-oxide isolated from the alkaloidal mixture, support the conclusion that the latter two compounds were principally—if not solely—responsible for the tumor inhibitory activity of the alcoholic extract of *S. triangularis*. The present report appears to be the first recorded observation of the inhibitory activity of pyrrolizidine alkaloids against the Walker carcinosarcoma 256 tumor (intramuscular), although the activity of monocrotaline against the adenocarcinoma 755 tumor has been noted earlier (7).

When the isolation procedure of Koekemoer and Warren (8) was used (*i.e.*, partition of total extract between chloroform and 15% citric acid solution, and reduction of *N*-oxides in the aqueous layer with zinc and hydrochloric acid), the yield of total alkaloid was 1.65% of weight of dry plant. Hence, *S. triangularis* appears to be one of the richest sources of pyrrolizidine alkaloids (*cf. References 1, 2*).

Characterization of senecionine (I) was effected by comparison of (*a*) the melting points of the alkaloid and its picrate and nitrate derivatives

[1] Rhizomes, roots, stems, leaves and flowers, gathered in Colorado, August 1961. 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 USDA by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health (CCNSC).

[2] Assays were performed by the Wisconsin Alumni Research Foundation and Hazleton Laboratories, Inc., under contract to the CCNSC. The procedures were those described in *Cancer Chemotherapy Rept.*, **25**, 1(1962).



Flow Sheet for the Isolation of Tumor-Inhibitory Principles from S. triangularis
Scheme I

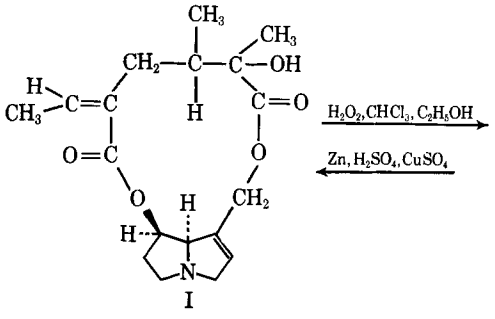
and (*b*) the specific rotation and infrared and NMR spectra with recorded data (9–12). In addition, hydrolysis with barium hydroxide solution afforded retronecine (III) (isolated as hydrochloride) and senecic acid (IV), characterized by comparing melting points and spectra with recorded data (9, 13).

Senecionine *N*-oxide (II) was crystallized from chloroform–petroleum ether as a chloroform solvate, m.p. 141–142° dec., $[\alpha]_D^{28}$ −22° (c 1.32, $CHCl_3$), $\lambda_{max.}^{Nujol}$ 5.73, 5.81, 6.04 μ.[3]

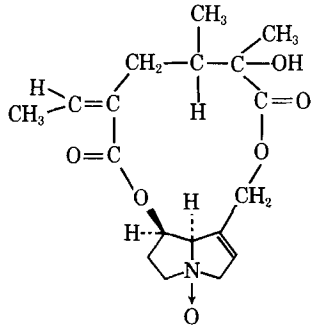
Anal.—Calcd. for $C_{18}H_{25}NO_6 \cdot {}^2/_3\ CHCl_3$: C, 52.02; H, 6.00; Cl, 16.45; N, 3.25. Found: C, 51.52; H, 6.24; Cl, 17.64; N, 3.55.

The compound was characterized by reduction of II with zinc, copper sulfate, and sulfuric acid to I, and by oxidation of I with hydrogen peroxide to II (14).

The NMR spectrum of senecionine *N*-oxide is similar to that of senecionine, except that the signals corresponding to the protons on carbon atoms adjacent to the nitrogen are shifted downfield by 0.6 to 1.2 τ units. Thus, the signal for the C-8 proton, which appears as a multiplet centered at 5.68 τ in the spectrum of senecionine (15), is shifted to 4.50 τ in the spectrum of the



I

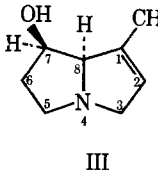


II

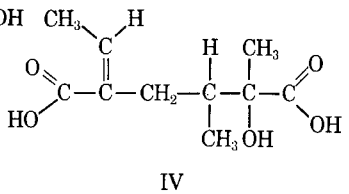
[3] Senecionine *N*-oxide is very hygroscopic; the crystals were filtered in a "dry box" and dried under reduced pressure at 60°.

TABLE I—ACTIVITY OF FRACTIONS OF *S. triangularis* IN WALKER CARCINOSARCOMA 256 (INTRAMUSCULAR)

Fraction	Dose, mg./Kg.	Survivors	Animal Wt. Change Diff., Gm. (T-C)	Tumor Wt., Gm. (Test/Control)	T/C × 100
A	210	4/4	−11	1.5/6.6	22
	140	4/4	−6	3.0/6.6	45
	93	4/4	−5	3.9/6.6	59
B	360	4/4	+4	6.7/6.6	101
	180	4/4	−5	7.6/6.6	115
	90	4/4	0	6.7/6.6	101
C	500	4/4	+2	5.2/6.6	78
	250	4/4	−2	6.3/6.6	95
	125	4/4	−2	7.1/6.6	107
D	30	2/4	−10	1.8/4.4	...
	25	4/4	−3	1.3/7.8	16
	20	4/4	−7	1.7/7.8	21
	15	4/4	+6	4.5/7.8	57
	10	4/4	−7	5.8/6.6	87
	5	4/4	−3	4.4/6.6	77
E	80	0/4	...	...	...
	40	4/4	−10	2.7/5.7	47
	20	4/4	0	3.0/5.7	52
F	50	3/4	−4	1.8/7.0	25
	40	4/4	−4	1.9/9.0	21
	30	4/4	−2	3.5/7.0	50
	20	4/4	−4	5.7/7.8	73
	10	4/4	−2	6.8/7.8	87



III



IV

N-oxide. The signals for the C-3 protons [multiplets at 6.14 and 6.53 τ for senecionine (15)] appear as a strong (2-proton) multiplet at 5.45 τ in the spectrum of the *N*-oxide. The shifts for protons on carbons adjacent to the nitrogen in the *N*-oxide are noteworthy, and may be valuable in structural studies of amines and their corresponding *N*-oxides.

It is noteworthy that various species of *Senecio* have been used in the treatment of cancer in many countries (16–19).[4] Indeed, *Senecio vulgaris*, known to contain senecionine (2), has been used since the 4th century A.D. for "tumors of the feet and sinews" (18), and since the 10th century for cancer (19). Furthermore, several other genera known to contain pyrrolizidine alkaloids [*e.g.*, *Petasites*, *Cynoglossum*, *Echium*, *Heliotropium*, *Tournefortia*, and *Cytisus* (2)] have also been described in folklore as plant remedies for cancer (16).

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Received December 23, 1966.
Accepted for publication February 13, 1967.

This investigation was supported by grant CA-04500 from the National Cancer Institute, grant T-275 from the American Cancer Society, and a contract PH 43-64-551 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
Previous paper: Kupchan, S. M., Hemingway, R. J., Knox, J. R., Barboutis, S. J., Werner, D., and Barboutis, M. A., *J. Pharm. Sci.*, to be published.

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