## Cardiotonic Activity in Callus Tissue of *Digitalis mertonensis*

*Sir:*

It is well known that the quantitative estimation of digitalis glycosides by chemical methods does not always indicate an equivalent biological effect. There are many glycosides that do not produce a biological effect that are measured in chemical assays. Bioassay is therefore still preferred to the chemical assay when estimating the activity of cardiotonic glycosides (1–3).

Static cultures of callus tissue of *D. mertonensis* Buxton and Darlington seedlings were grown in the dark at 27° ± 1° in White's (4) modified basic media to which ingredients suggested by Staba (5) were added. To induce formation of callus in 14-day-old seedlings, 2,4-dichlorophenoxyacetic acid and 2-benzothiazoleoxyacetic acid in 1 and 2 p.p.m., respectively, were used. Thirty-seven-day-old callus tissue was harvested and dried, and a tincture was prepared from it according to the U.S.P. (6) method for digitalis leaf.

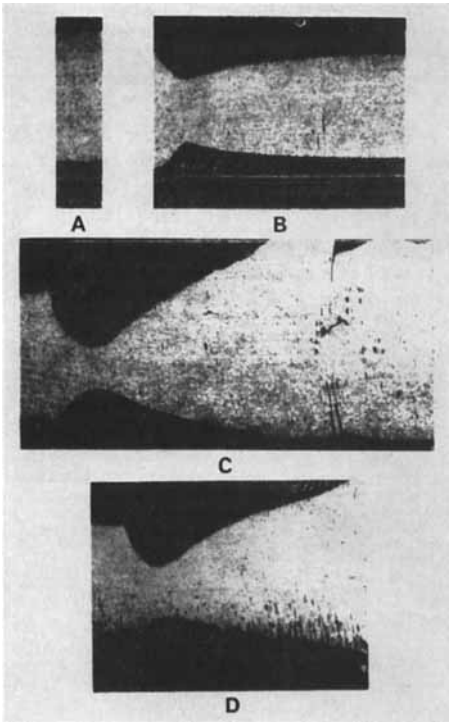


*Fig. 1—Recordings of an isolated guinea pig heart preparation on administration of U.S.P. standard digitalis tincture and* D. mertonensis *callus tincture* $IB_{2}c$. *Key:* A, *normal recording;* B, *effect of 0.25 ml. of 20% alcohol;* C, *effect of 25 mcg. total glycosides in diluted tincture of* $IB_{2}c$ *injected in 3 graded doses over a period of 8 min. (note + inotropic response);* D, *effect on same preparation 1.5 min. following injection of 33 mcg. of total glycosides in diluted standard tincture.*

Isolated guinea pig heart preparations were used to study the cardiotonic activity of the callus. Guinea pigs of either sex weighing from 500 to 900 Gm. were used. The isolated mammalian heart technique was basically that of Langendorff (7). The perfusion fluid used was that suggested by Chenoweth and Koelle (8) slightly modified by using magnesium sulfate in place of magnesium chloride (9). Following cervical dislocation, the hearts were quickly removed and suspended in an Anderson-Craver heart perfusion apparatus (10). When the isolated heart, under a perfusion head pressure of 54 cm. of water and at a constant temperature of 37.5°, became stabilized, all extraneous tissues including the pericardium were dissected free. A stainless steel hook was inserted into the apex. The cardiac contractions were recorded on a slowly moving smoked kymograph by means of a cotton thread running from the hook, through a pulley system to a recording lever. All perfusate was discarded.

The tinctures were diluted sufficiently to reduce the concentration of alcohol to less than 25%. After recording the normal cardiac contractions, the effect of 0.25 ml. of 20% alcohol was recorded as a control. This was followed by equivalent doses of test sample. In each case the test sample was completely washed in by displacing the liquid in the injection spiral (1.5 ml.) with fresh perfusate. When the heart returned to normal, a diluted standard digitalis tincture was also introduced and its effect recorded. The results were then compared. (Fig. 1.)

It was found that the callus tincture produced a typical digitalis-like response in the isolated guinea pig heart. There was an increase in amplitude with a dose of 25 mcg. of total glycosides[1] over a period of 8 to 10 min. In four separate trials this effect was comparable to an increase in amplitude produced by 333 mcg. of total glycosides[1] in the standard digitalis tincture. However, in terms of dry callus 37.5 mg. was

[1] The total glycosides in the callus tincture and the standard digitalis tincture were calculated as digitoxin based on the Knudson and Dresbach colorimetric assay for total digitalis glycosides (11).

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.

(1) Imai, S., Murase, H., Katori, M., Okada, M., and Shigei, T., *Japan. J. Pharmacol.*, **15**, 62(1965).
(2) Kohli, J. D., and Vohra, M. M., *Arch. Intern. Pharmacodyn.*, **126**, 412(1960).
(3) Vohra, M. M., Kohli, J. D., and De, N. N., *ibid.*, **133**, 265(1961).
(4) White, P. R., "The Cultivation of Animal and Plant Cells," 2nd ed., Ronald Press, New York, N. Y., 1963, pp. 60–61.
(5) Staba, E. J., *J. Pharm. Sci.*, **51**, 249(1962).
(6) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, pp. 189–190.
(7) Langendorff, O., *Arch. Ges. Physiol.*, **61**, 291(1895).
(8) Chenoweth, M. B., and Koelle, E. S., *J. Lab. Clin. Med.*, **31**, 600(1946).
(9) Smookler, H. H., and DeFeo, J. J., *J. Pharm. Sci.*, **51**, 736(1962).
(10) Anderson, F. F., and Craver, B. N., *J. Pharmacol. Exptl. Therap.*, **93**, 135(1948).
(11) Knudson, A., and Dresbach, M., *ibid.*, **20**, 205(1922).
(12) Medora, R. S., Ph.D. Thesis, University of Rhode Island, Kingston, R. I., 1965.
(13) Lendle, L., in "Handbuch der Experimentellen Pharmakologie," Heffter, Heubner, and Shuller, eds., Erganzungswerk, Springer, Berlin, Germany, 1935, vol. 1, pp. 11–265.

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[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).