combination of the ion pair IV by attack of the *p*-amino function on the three-membered heterocyclic ring leads directly to the zwitterionic form of the amino acid V. Decarboxylation of V is then unexceptional, being facilitated, in fact, by the presence of the *p*-amino group (5).

Finally, it seems pertinent to point out that this scheme might be substantiated by at least two methods. On the one hand, a salt corresponding to IV could conceivably be prepared and its decomposition products studied; and, on the other hand, advantage could be taken of the fact that in IV the two methylene groups in the three-membered ring lose their identity in contrast to their behavior in Scheme II*b*. Thus, the pyrolysis of a suitably labeled sample of I would be critical.

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R. A. FINNEGAN

Department of Medicinal Chemistry
School of Pharmacy
State University of New York at Buffalo

Received February 1, 1965.
Accepted for publication March 27, 1965.

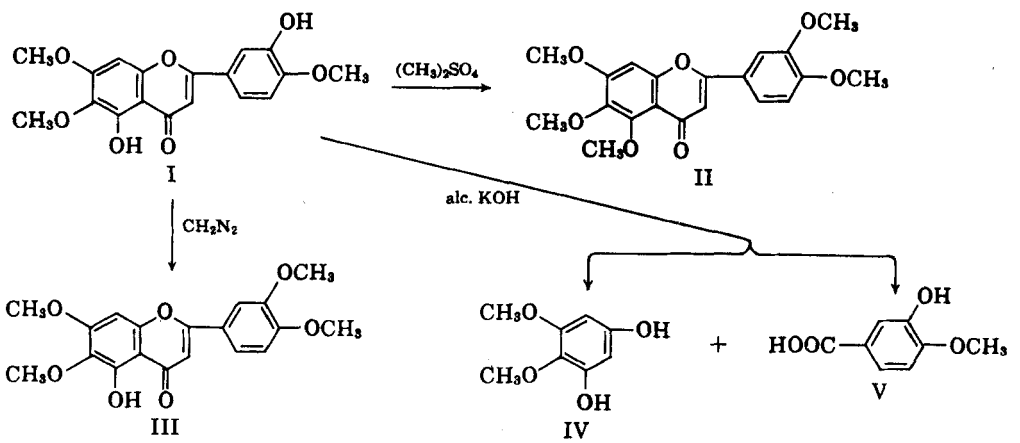
Tumor Inhibitors VIII. Eupatorin, New Cytotoxic Flavone from *Eupatorium semiserratum*

Sir:

In the course of our continuing search for tumor inhibitors from plant sources, an alcoholic extract of *Eupatorium semiserratum* DC[1] was found to have reproducible 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 structure elucidation of a new cytotoxic flavone, eupatorin.

The preliminary fractionation of the alcoholic extract (A) is summarized in Fig. 1. The mixed flavonoid band (H) was divided into sodium carbonate-soluble (I, 16.2 Gm.), sodium hydroxide-soluble (J, 20.6 Gm.), and nonacidic (K, 10 Gm.) fractions by partitioning with chloroform. Fraction J was chromatographed on silicic acid, and a band eluted by 5% methanol in chloroform was fractionally crystallized from ethyl acetate and from dioxane–water to yield eupatorin (I), m.p. 196–198°, $\lambda_{max.}^{alc.}$ 243 mμ (ε 17,400), 254 mμ (ε 19,300), 274 mμ (ε 19,800), 342 mμ (ε 27,700).

The empirical formula, $C_{15}H_7O_4(OCH_3)_3$, was assigned for eupatorin on the basis of elemental and methoxyl analysis. Methylation of eupatorin with dimethyl sulfate yielded II, m.p. 175–176°, $\lambda_{max.}^{alc.}$ 240 mμ (ε 25,900), 265 mμ (ε 16,500), 328 mμ (ε 28,600), characterized by comparison of its physical properties with those reported for 3′,4′,5,6,7-pentamethoxyflavone (1). Methylation of eupatorin with diazomethane gave 5-hydroxy-3′,4′,6,7-tetramethoxyflavone,



[1] Stems, leaves, and flowers were gathered in Florida, September 1963. 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 developed with the U.S.D.A. 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 Rept.*, **25**, 1(1962).

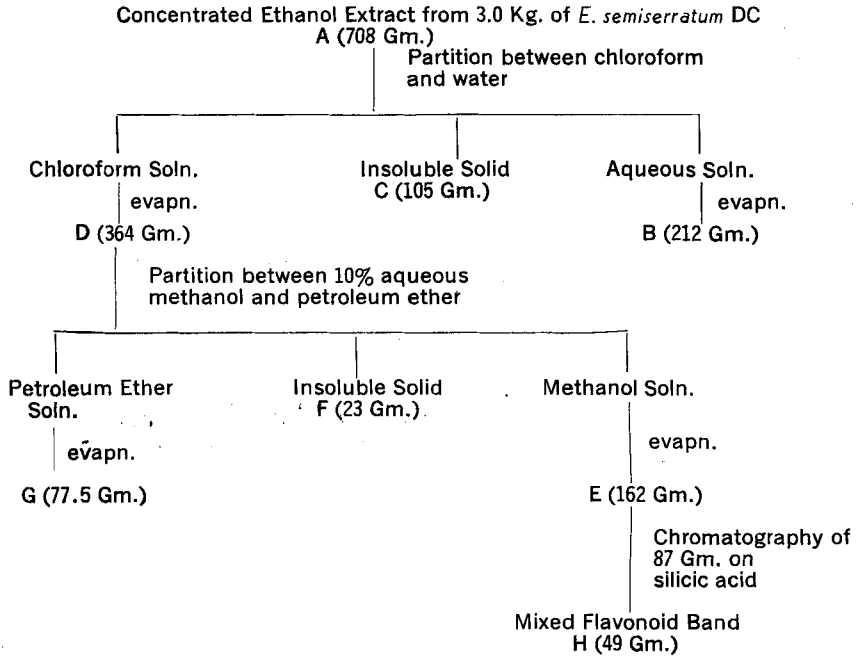


Fig. 1.—Flow sheet for fractionation of cytotoxic extract from *E. semiserratum*.

TABLE I.—CYTOTOXICITY OF FRACTIONS FROM *E. semiserratum*

Fraction	ED_{50} mcg./ml.	Fraction	ED_{50} mcg./ml.
A	2.9	G	>100
B	3.2	H	7.6
C	14	I	7.4
D	3.2	J	4.7
E	18	K	2.6
F	1.4	Eupatorin	2.5, 4.2

m.p. 189–190°, $\lambda_{max.}^{alc.}$ 242 mμ (ε 20,400), 275 mμ (ε 19,400), 339 mμ (ε 27,500), identified by direct comparison (mixed melting point, infrared spectrum) with an authentic sample[3] (2). The structural problem which remained at this point was the location of the second free hydroxyl group in eupatorin. Treatment of I with 50% 1:4 aqueous ethanolic potassium hydroxide under reflux gave 4,5-dimethoxyresorcinol (IV), m.p. 76–77°(113° after drying *in vacuo*) (3), and isovanillic acid (V). The structure of eupatorin was thus established as 3′,5-dihydroxy-4′,6,7-trimethoxy flavone (I).

The biological assay data (Table I) indicate that eupatorin is responsible for only a small fraction of the cytotoxicity of the extract of *E. semiserratum*. Studies of other cytotoxic constituents are in progress and will be reported in due course.

[3] The authors thank Professor Naokata Morita, University of Toyama, Toyama, Japan, for an authentic sample of 5-hydroxy-3′,4′,6,7-tetramethoxyflavone.

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S. MORRIS KUPCHAN
JOHN R. KNOX
M. S. UDAYAMURTHY

Department of Pharmaceutical Chemistry
University of Wisconsin
Madison

Received March 23, 1965.
Accepted for publication April 5, 1965.

This investigation was supported by grant CY-4500 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md., grant T-275 from the American Cancer Society, and contract PH43-64-551 from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health.