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# **Tumor Inhibitors 114 Aloe Emodin: Antileukemic Principle Isolated From Rhamnus frangula L**

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## **Abstract**

A systematic fractionation of an ethanol-water (1:1) extract of the seeds of *Rhamnus frangula* L., guided by assays for tumor-inhibitory activity, led to the isolation of Aloe emodin (1). This compound was found to show significant antileukemic activity against the P-388 lymphocytic leukemia in mice. A noteworthy vehicle-dependence of the testing results is reported. In the light of this vehicle-dependence, the re-examination of other anthraquinone derivatives is recommended.

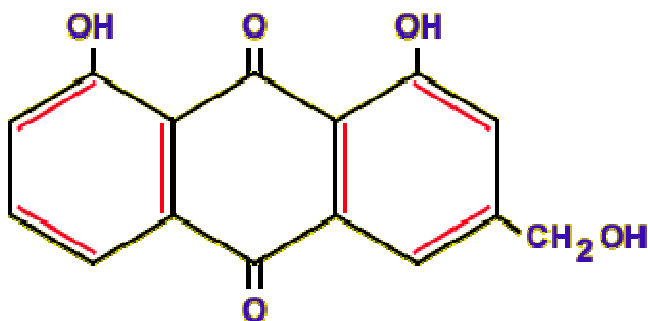
*Rhamnus frangula* L. (Rhamnaceae) has been used in England and the United States to treat cancers, and other *Rhamnus* species have been used similarly in folk medicine from at least the time of Galen (circa A.D. 150) (2).

In the course of our continuing search for tumor inhibitors of plant origin, an ethanol-water (1:1) extract of the seeds of *Rhamnus frangula* L.<sup>2</sup> showed significant inhibitory activity when tested in mice against the P-388 lymphocytic leukemia<sup>3</sup>. Fractionation of the extract, guided by assay against the P-388 system, revealed that the inhibitory activity was concentrated in the aqueous layer of a petroleum ether-water partition, and that the active material was extractable by chloroform from the aqueous solution. Column chromatography of the chloroform solubles on SilicAR CC-7 with 2.5% methanol in chloroform led to the isolation of Aloe emodin (1) from the active chromatographic fraction. The compound was characterized by direct comparison of its melting point, tlc, and infrared spectral characteristics with those of an authentic sample of Aloe emodin.



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Aloe emodin (1) shows significant inhibitory activity against the P-388 leukemia in mice when administered as a suspension in acetone-Tween 80. Results corresponding to T/C values of 133-154% were found at the optimal dose of 20 mg/kg.



In a recent review, the results of antitumor assays of 379 anthraquinone derivatives were reported. The authors concluded that “the most noteworthy observation concerning the anthraquinones is the relative lack of activity among the numerous derivatives tested from this group” (4). None were found to inhibit the L-1210 leukemia in mice, and only five showed some activity against solid tumor systems. Aloe emodin (NSC-38628) was among the derivatives which were found to be inactive. Since the P-388 system did not number among the tumors used in the study, our discovery of the antileukemic activity of Aloe emodin may reflect only a unique sensitivity of this mouse leukemia toward the compound. We note here, however, that the antileukemic activity of Aloe emodin is particularly vehicle-dependent, and that the reproducible inhibitory activity toward the P-388 system was manifested only when the acetone-Tween 80 suspension was used. In view of this fact, a re-examination of other anthraquinones for potential antitumor activity, with particular attention to possible vehicle-dependence, may be rewarded by the discovery of new and useful structure-activity relationships.

## Experimental

### Extraction & Fractionation -

Ground, dried seeds of *Rhamnus frangula* L. (1 kg) were extracted with ethanol-water (1:1, 7 liters) at room temperature overnight. The extract was filtered, concentrated under reduced pressure to about 1.5 liters and freeze-dried, to yield 163 g of residue. The residue was partitioned between petroleum ether (2 liters) and water (2 liters), whereupon 13.5 g of solid remained undissolved and was separated by filtration. Evaporation of the petroleum ether to dryness under reduced pressure yielded 11 g of residue. The aqueous solution was extracted with chloroform (2 X 2 liters), and evaporation of the chloroform extract to dryness under reduced pressure yielded 9.5 g of residue (fraction A).



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## Chromatography Of Fraction A -

A solution of fraction A (8 g) was treated with 25 g of SilicAR CC-7. The suspension was evaporated to dryness on a rotary evaporator, and the residue was added to a column of SilicAR CC-7 (500 g) prepared as a suspension in chloroform. The column was eluted first with chloroform (1 liter) and then with 2.5% methanol in chloroform, and 30 X 100 ml subfractions were collected. Subfractions were examined by tlc and those which were similar were combined and submitted for biological testing. The aggregate of subtractions 17-25, all rich in Aloe emodin ( $R_F$  0.54), constituted the sole active fraction (B, 1.9 g).

## Isolation Of Aloe Emodin (1) -

Active fraction B (1.5 g) was crystallized from chloroform-methanol, and recrystallization from the same solvents yielded orange-yellow needles (700 mg), mp 223-224°; lit. mp 223-225° (5). The melting point was not depressed by admixture of an authentic sample of Aloe emodin<sup>5</sup>. Mixture tlc and infrared spectral comparisons confirmed the identity of the two samples.

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## LITERATURE CITED

1. **Kupchan SM** 1976. Novel plant-derived tumor inhibitors and their mechanisms of action. *Cancer Chemother. Rep.*, in press.
2. **Hartwell JL** 1971. Plants used against cancer. A survey. *Lloydia* 34: 103.
3. **Geran RI; Greenberg NH; Macdonald MM; Schumacher AM; Abbott BJ** 1972. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (third edition). *Cancer Chemother. Rep.*, Part 3. 3: 1. Evaluation of assay results on a statistical basis in sequential testing is such that a material is considered active if it causes an increase in survival of treated animals (T) over controls (C) resulting in T/C >125 percent.



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4. **Driscoll JS; Hazard GF, JR.; Wood HB, JR.; Goldin A** 1974. Structure-antitumor activity relationships among quinone derivatives. *Cancer Chemother. Rep.*, Part 2, 4(2): 1.

5. **Karrer W** 1958. Konstitution und Vorkommen der organischen Pflanzenstoffe. *Birkhäuser Verlag*, BaSel. p. 517.

<sup>1</sup> Part 113 is reference 1.

<sup>2</sup> Seeds of *Rhamnus frangula* L. were collected in Austria in November, 1966. We acknowledge with thanks receipt of the dried plant material from Dr. R. E. Perdue, Jr., U.S. Department of Agriculture, in accordance with the program developed by the National Cancer Institute. Voucher specimens are on deposit at the Medicinal Plant Resources Laboratory, Agricultural Research Service, Beltsville, Maryland.

<sup>3</sup> Antileukemic activity was assayed under the auspices of the National Cancer Institute, by the procedure described in reference 3.

<sup>4</sup> Melting points were determined with a Mettler FP2 hot-stage microscope. Infrared spectra were determined with a Perkin-Elmer Hitachi model 257 spectrophotometer as KBr pellets. Petroleum ether refers to the fraction of bp 60-68°. Thin-layer chromatography was carried out on silica gel 60 F-254 (E. Merck) precoated plates, and chromatograms were visualized by spraying with an anisaldehyde-sulfuric acid spray; the developing solvent was 5% methanol in chloroform.

<sup>5</sup> We thank Professor H. Wagner, Universität München, for an authentic sample of Aloe emodin.