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CENTAUREIDIN, A CYTOTOXIC FLAVONE FROM *Polymnia fruticosa*, INHIBITS TUBULIN POLYMERIZATION

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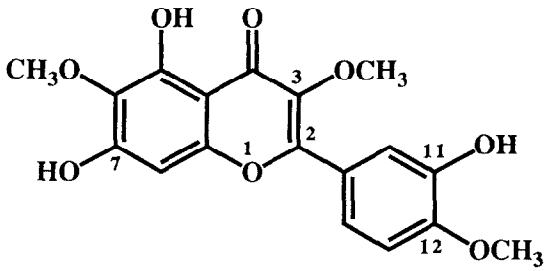
ABSTRACT: The characteristic pattern of differential cytotoxicity of a crude extract of the tropical plant *Polymnia fruticosa* was found to be similar to those of known tubulin-interactive compounds. Fractionation of the extract led to centaureidin as the major cytotoxic principle. Centaureidin inhibited tubulin polymerization, inhibited the binding of [^{3}H]-colchicine to tubulin, and induced mitotic figure formation in whole cells at cytotoxic concentrations. This is the first known example of a flavone with antimitotic activity.

The NCI in vitro human disease-oriented tumor screening panel was designed, in part, to detect compounds with characteristic patterns of differential cytotoxicity toward the various solid tumor lines which comprise the panel[1,2]. Compounds with similar mechanisms of action or biochemical targets typically yield similar patterns or screening "fingerprints" in the assay. Thus, when compounds which are known to act through a tubulin-dependent mechanism are tested, the resulting profiles of differential cytotoxicity are similar with regard to which cell lines are more sensitive or more resistant to the drugs. To fully exploit these observations, a computerized pattern-matching algorithm, which compares the screening "fingerprint" of a selected "seed" compound or extract to the databases of extracts, compounds, or fractions which have been tested to date in the assay, has been developed[2,3].

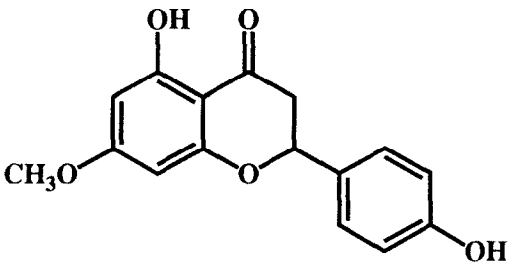
The crude differential cytotoxicity profile of the organic extract from the plant *Polymnia fruticosa* Benth. (Compositae), showed consistently high correlations with the profiles of known tubulin-interactive, antimitotic reference compounds (e.g., vincristine, maytansine, taxol, rhizoxin, dotastatin 10). Therefore, the organic extract (2.5 g) of *P. fruticosa* was subjected to bioassay (cytotoxicity)-directed fractionation to isolate the active constituent(s). Initial solvent-solvent partitioning gave a cytotoxic $CHCl_3$ fraction. Gel permeation of this fraction through Sephadex LH-20 in CH_2Cl_2-MeOH (1:1) gave an active fraction (mean

GI_{50} 0.83 μg/ml) which was purified by gradient HPLC on a cyano-bonded phase to give centaureidin (**1**, 10 mg, mean GI_{50} 0.27 μg/ml). Our isolate was identified as centaureidin by comparison of spectral data with those reported in the literature[4-7]. First elucidated by Farkas *et al.*[4], this flavone was later identified as a cytotoxic toward KB cells by Kupchan[5] and Jeffries *et al.*[6]. It has been reported as a constituent of 22 other genera of the Compositae. We have reversed the NMR assignments reported by Parodi *et al.*[7] for two pairs of carbons (C-2/C-7 and C-11/C-12), based on the results of HMBC and HBQC experiments.

Two other compounds previously reported from this species by Bohlmann *et al.*[8], sakuranetin (**2**) and the cinnamate ester of 3-(p-hydroxyphenyl)-1-propanol, were also isolated during purification of **1**. Neither of these latter compounds was markedly cytotoxic. It is notable that the profiles of differential cytotoxicity of the pure centaureidin (Figure 1), the active fractions containing **1**, and the crude extract were highly correlated throughout, demonstrating that patterns of differential cytotoxicity are consistently detectable during fractionation.



1 centaureidin



2 sakuranetin

Results of biochemical testing of the pure centaureidin were consistent with a tubulin-interactive, antimitotic mechanism of cytotoxicity. In a turbidimetric assay of tubulin polymerization[9], centaureidin blocked the extent of the reaction by 50% at a concentration of 3 μM (Figure 2); in the same assay, colchicine had an IC_{50} of 1.9 μM. Centaureidin (5 μM) also inhibited the binding of 5 μM [^{3}H]-colchicine to purified tubulin[10] at 37°C by 55%, compared to 5 μM podophyllotoxin, which was 84% inhibitory. Finally, centaureidin produced an increase in mitotic figures in CA46 Burkitt lymphoma cells at concentrations which were also cytotoxic to those cells (IC_{50} 0.2 μM, Figure 3). These results support the notion that centaureidin exerts its cytotoxic effect through interference with the polymerization of tubulin into microtubules.

While many other aromatic chemotypes are known to have similar effects (e.g., colchicine, vincristine, podophyllotoxin, combretastatins), flavones have not previously been identified as acting through a tubulin-

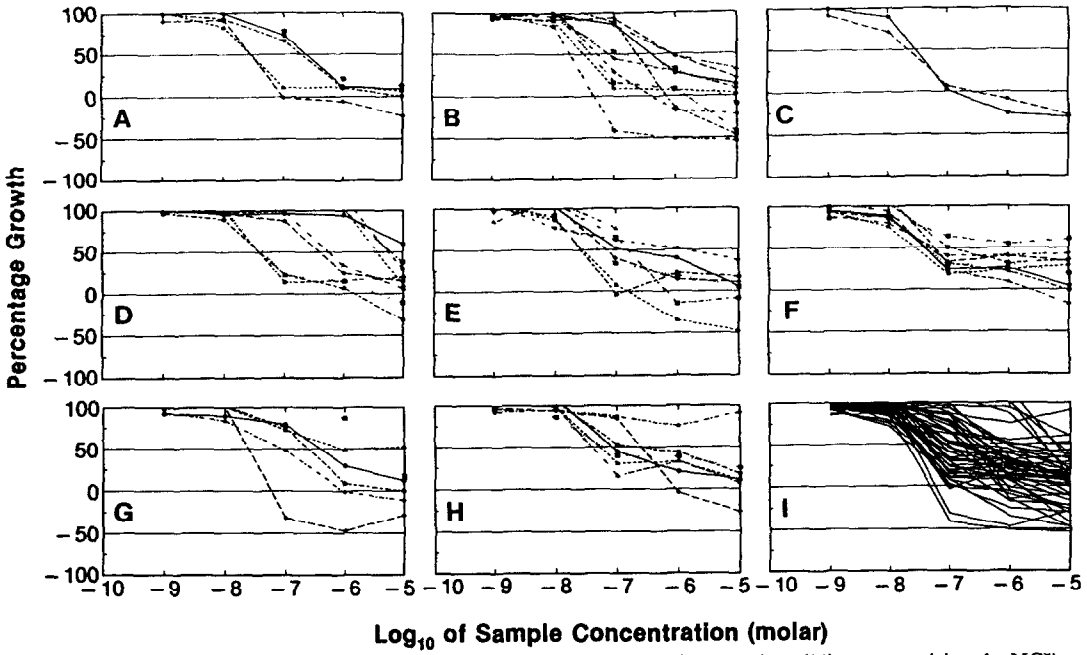


Figure 1. Dose response curves from the testing of centaureidin against the 60 cell lines comprising the NCI's human tumor disease-oriented in vitro screen. Individual cell line identifiers are omitted for clarity. Graph A is from the leukemia/lymphoma subpanel, graph B is the non-small-cell lung cancer subpanel, graph C the small-cell lung cancer subpanel, graph D the colon cancer subpanel, graph E the brain tumor subpanel, graph F the melanoma subpanel, graph G the ovarian cancer subpanel, graph H the renal cancer subpanel, and graph I the composite of the respective subpanels together.

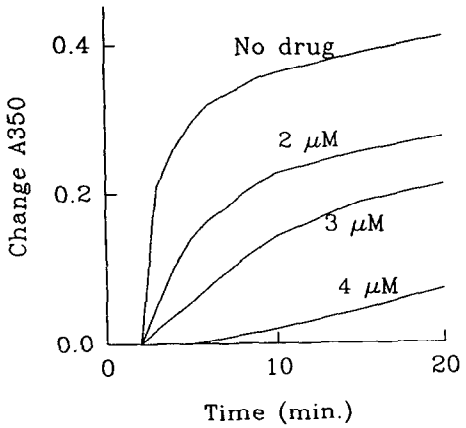


Figure 2. Turbidimetric study of tubulin polymerization affected by centaureidin.

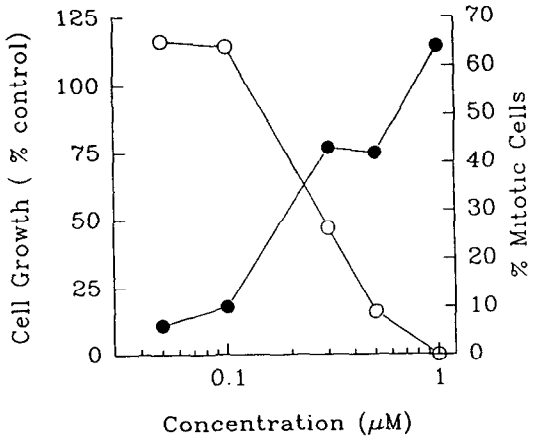


Figure 3. Cytotoxicity of centaureidin toward CA46 Burkitt lymphoma cells. Open circles, growth (36 hrs), closed circles, mitotic index (24 hrs).

dependent mechanism. Compare algorithm analyses of the screening data for several thousand plant extracts have revealed very few potential antimitotic leads. It would seem, therefore, that few members of the large class of flavones and flavanoids exhibit such activity. An investigation of the distribution of antimitotic activity in flavones is in progress and will be reported in due course.

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