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Foreword

The current year of activity in this portion of AP6 had the novel anticancer activity of extract SU 719 (grapefruit seed) as its main focus. Additional work included the general toxicity screening of 105 plant extracts and the initiation of HIV screening of those same extracts. Topoisomerase II (top2) is the target of some of the most useful anti-cancer drugs. All of the clinically used top2 drugs act by stabilizing a drug-enzyme-DNA cleavable complex. Here we report the activity of 719 a polycyclic phenol that possesses a new top2 mechanism of action. 719 is cytotoxic to cells. Yeast cells that over express top2 are more sensitive to 719 than cells that constitutively express top2. In addition, 719 possesses a unique top2 activity *in vitro*. It induces human top2 to catenate plasmid DNA in a purified enzyme system, while at the same time only producing minimal DNA cleavage. We hypothesize that this catenation correlates with the ability to aggregate DNA. These are activities shared with only one known compound, neoamphimedine, a pyridoacridine obtained form a marine organism discovered by us approximately a year ago. Neoamphimedine shows potent anti-neoplastic activity in human xenograft tumors in athymic mice and it is hypothesized that 719 will also. Significant progress toward structure determination has been accomplished with purification (>99%) of approximately 10 mg of active component and determination of the proton NMR spectrum and MW by positive and negative ion MS analysis.

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INTRODUCTION:

AP1 of the ICBG "Drug Development and Conservation of Biodiversity in West and Central Africa," provides data on the variety, distribution, abundance and dynamics of West and Central African flora. Other APs focus primarily on the chemistry and anti-parasitic activities of traditionally used medicinal plants. The research conducted under AP 6 is primarily concerned with development of African medicinal flora for their non-antiparasitic activities. Screening extracts from locally used plants can capitalize on traditional knowledge in order to provide information about the potential alternative uses of known plants. Random screening of West African flora for anticancer activity can provide information on the utility of species not recognized by traditional healers for diseases not regularly usually treated at that level.

BODY:

As described in our approved statement of work, our approach is to prioritize cytotoxic extracts based on screens that can detect cancer relevant mechanisms of tumor cell killing. We first test extracts for the ability to kill human cancer cells in culture. We then utilize mammalian cell based screens to detect molecules in the extracts that interfere with DNA metabolism or the cell cycle. In the mechanism screens cytotoxic extracts/molecules are tested for enhanced cytotoxicity in mutant cell lines that lack various cancer predisposition/tumor suppressor gene functions (caretaker functions). Cytotoxic extracts/molecules showing no DNA directed activity are tested by flow cytometry for the ability to disturb progression of normal cells through the cell cycle (tumor suppressor gatekeeper functions). These assays were described in previous reports. Follow up on this work is presented here.

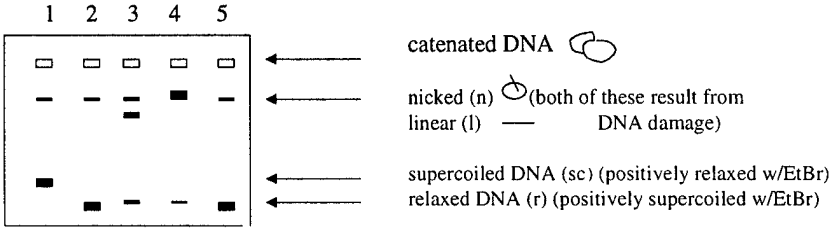
The principle focus of work this year was the grapefruit seed extract SU 719. Topoisomerase II (top2) is the target of some of the most useful anti-cancer drugs. All of the clinically used top2 drugs act by stabilizing a drug-enzyme-DNA cleavable complex. Here we report the activity of 719 a polycyclic phenol that possesses a new top2 mechanism of action. 719 is cytotoxic to cells. Yeast cells that over express top2 are more sensitive to 719 than cells that constitutively express top2, indicating that the effect is top2 mediated. In addition, 719 possesses a unique top2 activity *in vitro*. It induces human top2 to catenate plasmid DNA in a purified enzyme system, while at the same time only producing minimal DNA cleavage. We hypothesize that this catenation correlates with the ability to aggregate DNA. These are activities shared with only one known compound, neoamphimedine, a pyridoacridine obtained form a marine organism. Neoamphimedine shows potent anti-neoplastic activity in human xenograft tumors in athymic mice and it is hypothesized that 719 will also. Significant progress toward structure determination has been accomplished with purification (>99%) of approximately 10 mg of active component and determination of the proton NMR spectrum, shown below, and a MW of 449 by positive and negative ion MS analysis of the deuterated species.

Because of the activity SU 719 in DNA-damage sensitive cell lines and yeast, agarose gel analysis was used to visualize the reaction products of human topoisomerase in the presence of 719 in a purified enzyme system (Figure 1). This system used purified human topoisomerases I and II with supercoiled (circular) plasmid DNA as substrate. Analysis of the topoisomerase II reaction revealed a high molecular weight DNA product. Such a product has only been observed with a marine natural product that causes DNA aggregation *in vitro* and forces topoisoemrase II to work in reverse. Thus, instead of untangling and relaxing supercoiled DNA the enzyme actually interlinks the DNA circles to produced a large catenated complex.

DNA Catenation Assay

•Determines DNA cleavage and catenation, and whether cleavage or catenation is topo-dependent

•Pure enzyme system using supercoiled, ds 3H mp 19 plasmid DNA, as substrate, and agarose gel electrophoresis to resolve the products (see figure below)



•DNA conformation determined as nicked, linear, supercoiled and relaxed

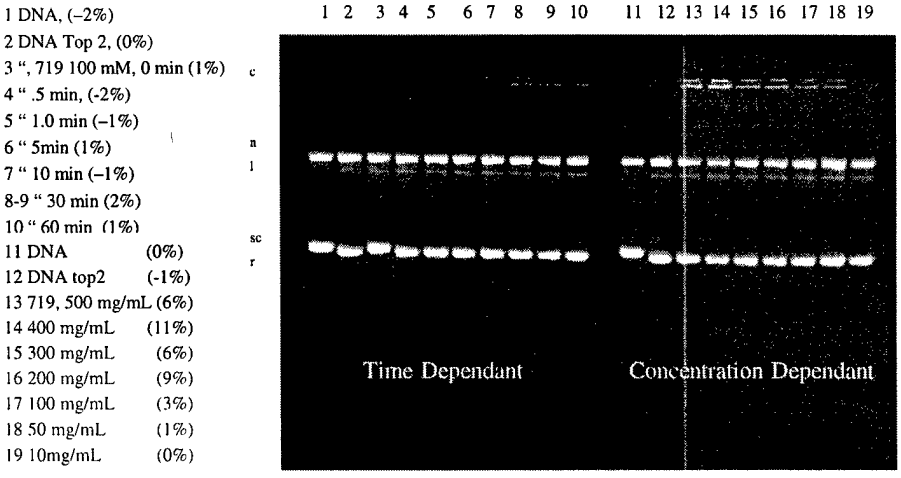
•Yield of DNA quantified through liquid scintillation counting

Figure 1. Agarose gel analysis of topoisomerse II and plasmid DNA reaction products.

Figure 2. Catenation of DNA by SU 719.

African Grapefruit Seed Extract (719)

*Catenates DNA in ethidium bromide gels



HPLC Tracing of 719

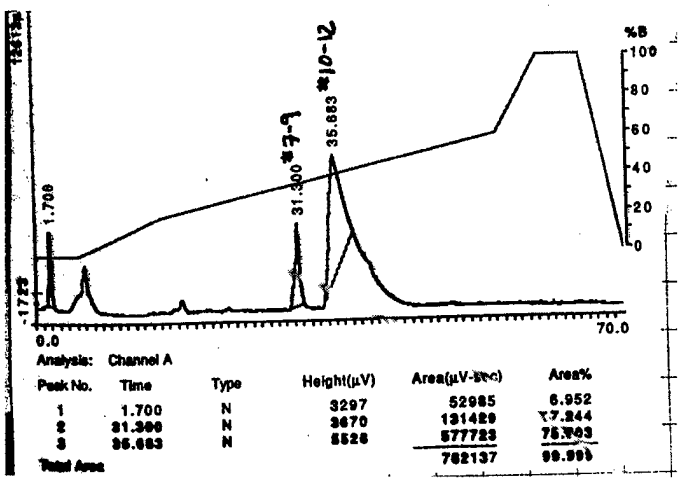
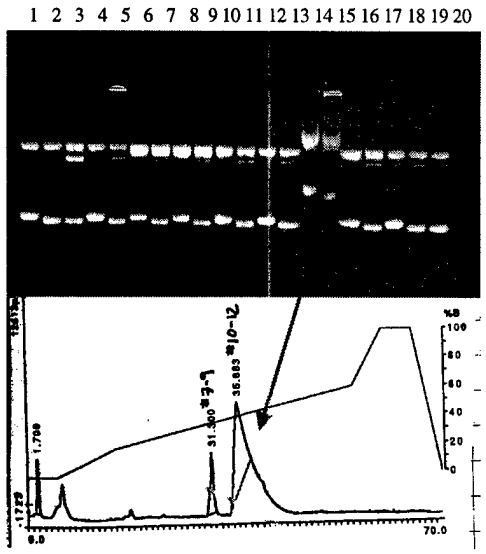


Figure 3. Reverse phase HPLC tracing of SU 719.

Figure 4. Topoisomerse II activity of SU 719 fractions.

Activity of HPLC fractions

	% Cleavage	% Catenation
1 DNA	4	–2
2 DNA top 2	0	0
3 100 uM Etop top 2	24	–2
4 719 500	6	–2
5 719 with top 2	-9	15
6 1	39	0
7 1 top 2	39	0
8 1-3	37	–1
9 1-3 top2	31	1
10 4-6	13	1
11 4-6 top2	16	1
12 7-9	9	–1
13 7-9	46	1
14 10-12	29	0
15 10-12 top2	9	29
16 13-15	16	0
17 13-15 top2	11	0
18 16-18	-6	-1
19 16-18 top 2	0	1
20 DNA top 2	-3	0



Yeast Sensitivity Assay

- Enhanced drug permeability of JN394 (T1+). T1+ is grown in rich media, JN362aT2 (Topo II over-expresser) in minimal media, overnight and counted in a hemocytometer, drug (in DMSO) is added
  - The Topo II over-expresser should show more sensitivity to a Topo II poison
- Cells are plated in duplicate onto rich agarose plates to determine clonal survival
- Cell survival is determined by normalizing the results to untreated (DMSO) to 100%

Yeast Sensitivity Data

	JN394	JN362aT2
DMSO	100	100
Etoposide 10 uM	21	5
Extract 1 ug/mL	31	24

Figure 5. Decreased survival of topoisomerse II overexpressing yeast cells to etoposide and 719.

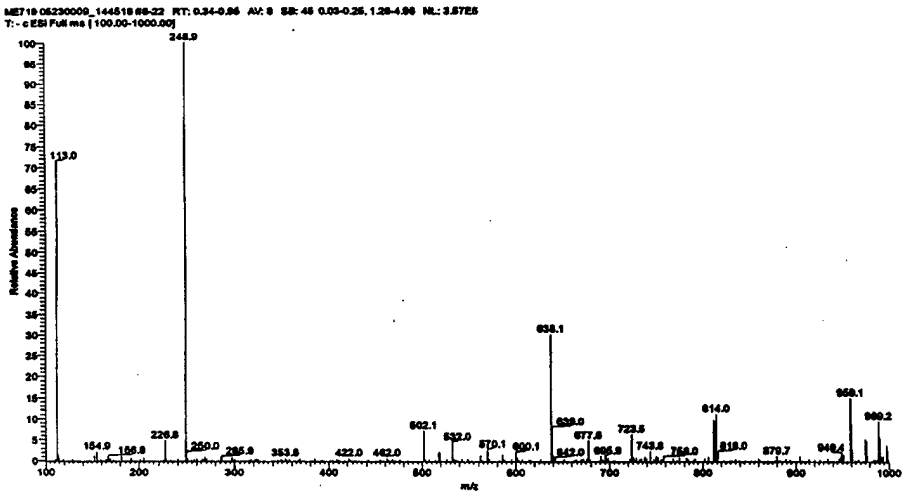


Figure 6. Negative ion MS spectrum of deuterated SU 719, MW appears to be 449.

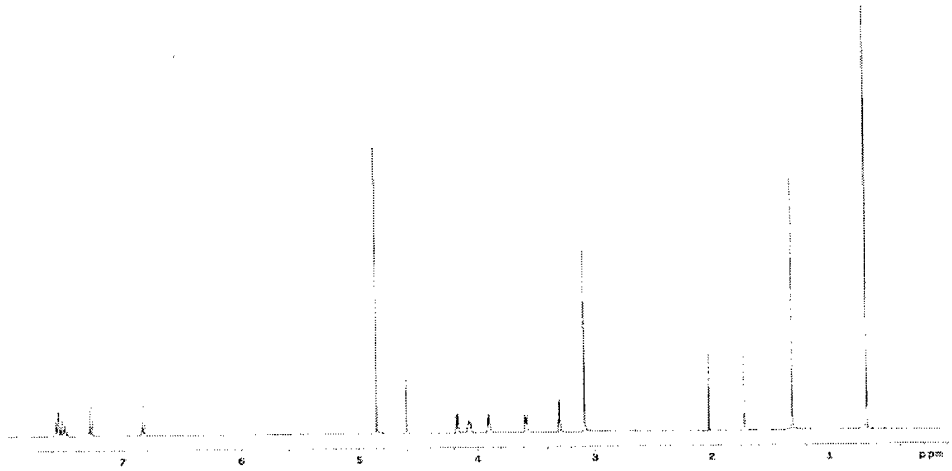


Figure 7. Proton NMR of SU 719

TABLE 1
Cytotoxicity of SU-2140 through SU-2473 in human KB cells

SU-2140 - -	SU-2169 - -	SU-2201 - -	SU-2231 - -	SU-2442 - +/-
SU-2141 - -	SU-2170 - -	SU-2202 - -	SU-2232 + +	SU-2443 - -
SU-2142 - +	SU-2171 - -	SU-2203 - +	SU-2233 - +	SU-2444 - -
SU-2143 - -	SU-2172 - -	SU-2205 - -	SU-2234 - -	SU-2445 - -
SU-2144 - -	SU-2173 - -	SU-2206 - +/-	SU-2236 +/-+	SU-2446 - -
SU-2147 - +	SU-2175 - -	SU-2207 - +/-	SU-2237 - +	SU-2447 - -
SU-2148 - -	SU-2176 - -	SU-2208 - +	SU-2238 - +	SU-2448 + +
SU-2149 - +	SU-2178 - -	SU-2211 - +	SU-2242 +/-+	SU-2449 - -
SU-2150 - -	SU-2179 - +/-	SU-2212 - +	SU-2278 - -	SU-2450 - +/-
SU-2151 - -	SU-2180 - -	SU-2213 - -	SU-2430 - +/-	SU-2451 - -
SU-2154 - -	SU-2181 - +/-	SU-2214 - -	SU-2431 - -	SU-2452 - +
SU-2155 - -	SU-2182 - -	SU-2215 - -	SU-2432 - +/-	SU-2453 - +
SU-2156 - +/-	SU-2183 - +	SU-2216 - -	SU-2433 - -	SU-2454 - -
SU-2157 - -	SU-2184 - -	SU-2217 +/-+/-	SU-2434 - -	SU-2455 ?
SU-2162 - +/-	SU-2186 - +	SU-2219 - -	SU-2435 - -	SU-2456 - -
SU-2163 - -	SU-2187 ?	SU-2220 - -	SU-2436 - +	SU-2457 - +/-
SU-2164 - +	SU-2189 - -	SU-2221 - -	SU-2437 - +	SU-2458 - -
SU-2165 - -	SU-2190 - +	SU-2224 +/-+	SU-2438 - -	SU-2459 - +/-
SU-2166 - -	SU-2191 - +	SU-2225 - +	SU-2439 - +	SU-2460 - -
SU-2167 - -	SU-2192 - +	SU-2226 - +	SU-2440 - +/-	SU-2461 - -
SU-2168 - -	SU-2200 - +/-	SU-2230 - +	SU-2241 - +	SU-2473 - +

KEY RESEARCH ACCOMPLISHMENTS:

- A unique cytotoxicity mechanism suggesting anticancer potential was identified in extract SU 719.
- Approximately 10 mg of the active component has been isolated at 99% purity.
- Structure identification has been initiated with determination MW and proton NMR spectrum.

REPORTABLE OUTCOMES:

- 105 extracts were screened for general cytotoxicity levels to complement on going anti-parasitic evaluation of other APs.
- A unique biological activity in extract SU 719 was identified and the active component isolated for structure determination.
- An AIDS Fogarty International Research Collaboration award, based on work described in last years annual report has been funded and is underway to initiate anti-HIV screening of African medicinal plant extracts.
- This research has supplied rotation projects and contributed to the training of two graduate (Ph.D.), and three undergraduate students at the University of Utah.

CONCLUSIONS:

Traditional medicines have been the source of several very important anticancer medicines (e.g., the vinca alkaloids, the podophyllotoxins and the camptothecins). The work in progress here has focused on a particular extract as containing a constituent with significant anticancer potential. Significant progress has been made towards identification of the molecular mechanism of this lead compound. The extract has been

fractionated and the active component isolated via chromatography and bioactivity guided isolation. The molecule possess the potential to combat some of the most serious diseases confronting mankind today, and as such possesses significant economic value if that potential is realized.