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A STUDY ON CYTOTOXIC PRINCIPLES OF SELECTED ANNONACEAE SPECIES

THESIS PRESENTED

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APPROVAL SHEET

A STUDY ON CYTOTOXIC PRINCIPLES OF SELECTED ANNONACEAE SPECIES

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CONTENTS

INTRODUCTION

Advances in the prevention and treatment of cancer will require the continued development of novel and improved chemotherapeutic agents. In the short history of the cancer drug development effort, a number of clinically useful agents have been developed by screening programs coordinated by the pharmaceutical industry[1-3]. Although chemotherapy for cancers with a high growth fraction has achieved important advances and resulted in improved cure rates for these diseases, little impact has been made on the solid human cancers such as lung, colon, breast, ovarian, prostate, pancreas and brain[2].

Natural products have a history of providing, novel chemically useful anticancer drugs, and a number of these have been coming from higher plants[4-6]. This situation suggests that new directions must be taken in the approach to discovery of drugs for these diseases. In response to this need, new screens designed to uncover agents specific for these forms of human disease have been initiated. The new screens are based on activity-directed fractionation of plant extracts, guided by *in-vitro* brine shrimp cytotoxicity[7,8] and/or testing on human tumor cell lines (HTCL)[1,2].

During the last 15 years several species of family Annonaceae have attained particular importance; where they proved to produce compounds with significant cytotoxic activity on human tumor cell lines *in-vitro* screens[9-12] (*viz*.acetogenins, diterpenes and others).

The present study is devoted for the investigation of the cytotoxic/antitumor principles in some selected Annonaceae species cultivated in Egypt. After screening of the different plant material, certain plants will be selected to enter a detailed systematic bioactivity-directed fractionation, aiming to the isolation and characterization of their bioactive principles.

REVIEW OF LITERATURE

1- Plants with cytotoxic activity:

General

Many new natural compounds are isolated, characterized, and published without any biological testing whatsoever. Their useful biological activities can remain unknown for years. Without accompanying biological data, the discovery of new medicinal plant constituents is nothing more than pure phytochemistry[7], of the most important areas that still deserve research activities are the plant-antitumor discovery programs. Because testing antitumor activity *in-vivo* is highly time consuming and expensive an *in-vivo* bioassay is required. Cytotoxicity of compounds and extracts has proved a useful property indicative for cancer chemotherapeutic activity[1].

There are specific methods for measuring cytotoxicity *in-vitro*, two of them proved very useful, these are:

- Brine shrimp cytotoxicity[7,8], which is a convenient general bioassay, and is also indicative for cytotoxicity. It determines the lethality of *Artemia salina* naupulii when exposed for 24 hr to the plant extract or fraction.
- Human tumor cell cytotoxicity assay (HTCC)[1,2], which is a more specific method and may involves five or more human tumor cell lines [*e.g.* A-549 lung carcinoma, HT-29 colon adenocarcinoma, U251-MG glioblastoma multiforme, RPMI-7951 Malignant melanoma and MCF-7 breast adenocarcinoma].

2- History of cytotoxic / antitumor compounds in plants:

Natural products have a history of providing a novel chemically useful anticancer drugs[1-3]. Specific examples are *Vinca* alkaloids, (vincristine, and vinblastine)[13], podophyllotoxins[14], colchicine derivatives[15],and taxol[16,17]. Other, work indicine-N-oxide, and homoharringt-

onine[1] have entered clinical trials. The scientific literature is rich with plants which possess variable efficacy of cytotoxic/antitumor activity[18-30]

Family Annonaceae has attained particular importance, and proved to produce compounds with significant cytotoxicity on HTCL. These compounds are acetogenins (*viz.* bullatacin, uvaricin .... etc)[31-85], alkaloids (*viz.* liriodenine, argentinine, roemarine and annoretine)[86-92], diterpenes (clerodan skeleton)[93, 94], and lignan (syringaresinol)[95].

3- Cytotoxic acetogenins in family Annonaceae

A- General

Annonaceae is a family of aromatic trees, shrubs or climbers, which grow in tropical and subtropical regions[9]. Annonaceae comprises *ca* 120 genera and more than 2000 species[96]. The plants exhibit a broad range of potent biological activities, [*viz.* cytotoxicity, antitumor, antimalarial, antimicrobial, antiparasitic, insecticidal, and immunosuppressant][9 -12]. Accordingly, several members of Annonaceae have attracted more and more attention[10]. Activity-directed fractionation of *Uvaria acuminata* examined by Jolad *et al.* (1982)[31], using 3ps (*in-vivo* murine leukemia), led to the isolation and structure elucidation of uvaricin an unusual antitumor compound. This is the first example of a new class of extremely bioactive compounds that are now referred to as annonaceous acetogenins[12]. The number of these acetogenins have reached now more than 230 compounds[77].

B- Structure [9-12]

Acetogenins are C35-C39 compounds and typically contain two long range hydrocarbon chains (*cf.* Fig. 1), one of which connects a terminal 2,4-disubstituted-γ-lactone to a variable number of tetrahydrofuran (THF) rings. The hydrocarbon chains contain a number of oxygenated moieties which can be hydroxyls, acetoxyl and/or double bonds, epoxides; compounds which lack THF rings, have been reported.

Acetogenins can be classified into four main groups according to the number and arrangement of the THF ring (s)[9,10,12].

- Adjacent bis THF annonaceous acetogenins[31-54]

They vary in the number of hydroxyl groups or carbons (C35 or C37) which they contain, the placement of bis - THF rings and hydroxyl groups on the chain e.g. uvaricin (*cf.* Fig 2).

- Non adjacent bis THF annonaceous acetogenins[55,56]

They contain two non adjacent THF rings, which are always separated by a four carbon chain. One THF ring is flanked by two hydroxyl groups while the other THF ring has only adjacent hydroxyl group which is positioned between the two THF rings e.g. bullatalicin (*cf.* Fig 2).

- Mono - THF annonaceous acetogenins [57-79]

These compounds are all characterized by the presence of a single THF, but different compounds vary in the number and placement of hydroxyl groups, the length of hydrocarbon chains (C37 to C39), the type of terminal γ-lactone, as well as the presence and placement of keto groups, double bonds and/or the mono-THF ring on the aliphatic chain e.g. annonacin (*cf.* Fig. 3).

- Non-THF annonaceous acetogenins (epoxy)[80-85]:

Recently α,β- unsaturated-γ-lactone compounds without THF rings have been isolated e.g. epoxyrollin A (*cf.*Fig. 3).

Since the classification of acetogenins is based on the number and arrangement of THF-rings, another subclass classification could be considered based on the terminal γ-lactone type as shown in Fig.(4).

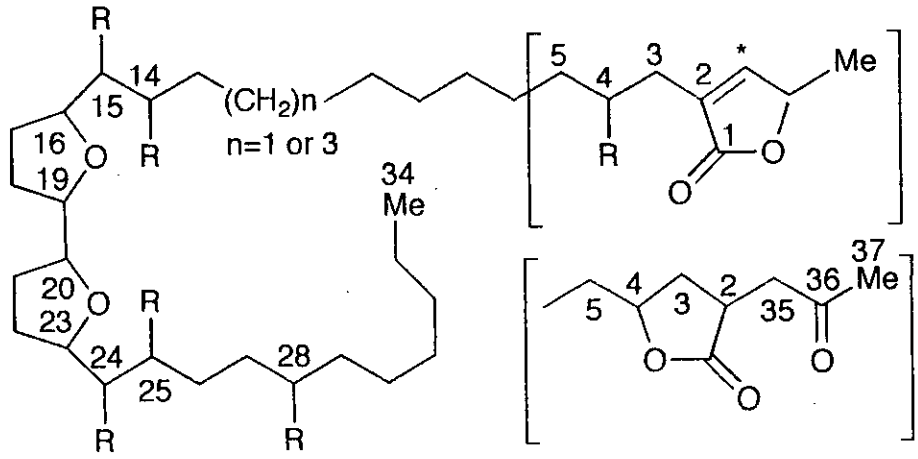
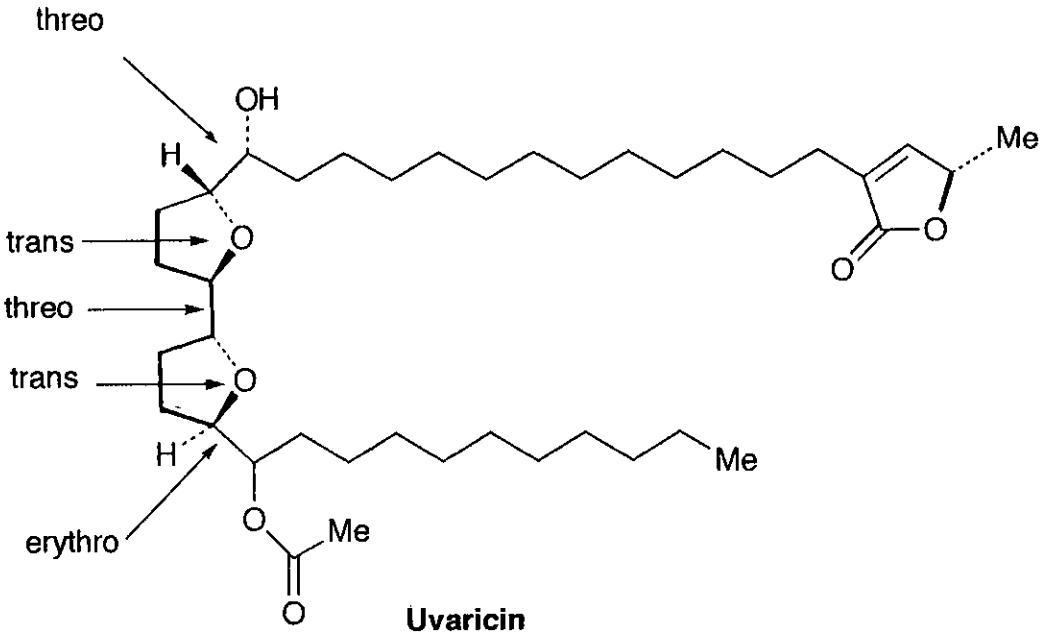


Fig. (1) : Adjacent bis-tetrahydrofuran ring acetogenin Carbon Skeleton[9]

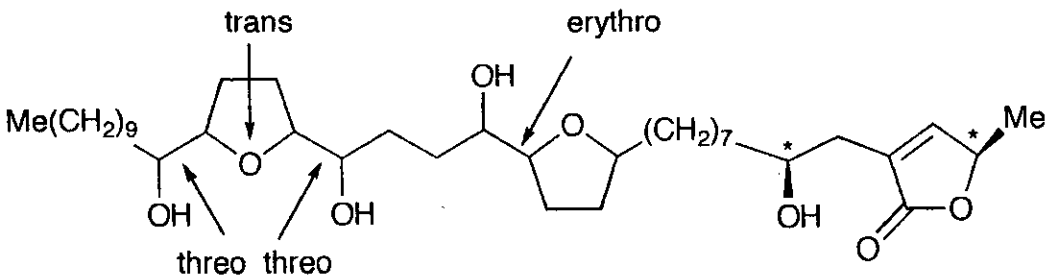
R represents either H, OH, ketone carbonyl, or acetoxyl.

The * indicates that the lactone ring may be saturated



Uvaricin

(1) Adjacent bis-tetrahydrofuran



Bulatalicin

(2) Non adjacent bis-tetrahydrofuran

Fig. (2) : The main skeleton of bis-tetrahudrofuran acetogenins