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# Chemistry and biological activities of *acetogenins from Annonaceae species*

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**ABSTRACT** A brief review on the chemical aspects and biological activity of *acetogenins* isolated from *Annonaceae* species which includes their detection, purification and structural identification, is presented.

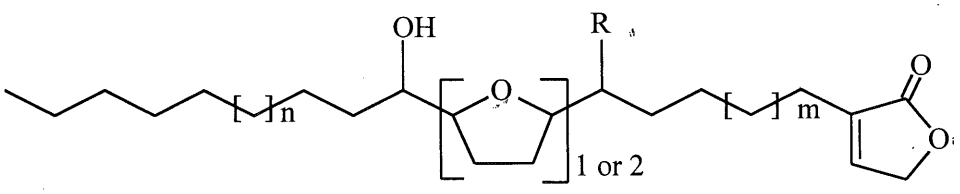
**ABSTRAK** Satu ulasan ringkas mengenai aspek-aspek kimia asetogenin dan keaktifan biologi dari sepsis dari *Annonaceae* yang merangkumi pengesanan, pencirian dan penentuan struktur dibentangkan.

(*Annonaceae, acetogenin*, FAB, NMR, *Annona muricata*)

## INTRODUCTION

In 1882, Jolad et al isolated uvaricin, a new antitumor agent, from the roots of *Uvaria acuminata* (Annonaceae) [1], a bis-tetrahydrofuranoid fatty acid lactone, related to polyketides or acetogenins. However, it presented some original structural characteristics, particularly a linear chain, the bis-tetrahydrofuran pattern flanked by two hydroxyls and a terminal unsaturated lactone. Two years later four new products presenting the same structural characteristics appeared in the literature, also isolated from Annonaceae [2,3]. Other similar compounds were isolated later.

Sine these products formed a new class of natural compounds and was only found in species belonging to the family of Annonaceae, they are commonly called "acetogenins from Annonaceae".



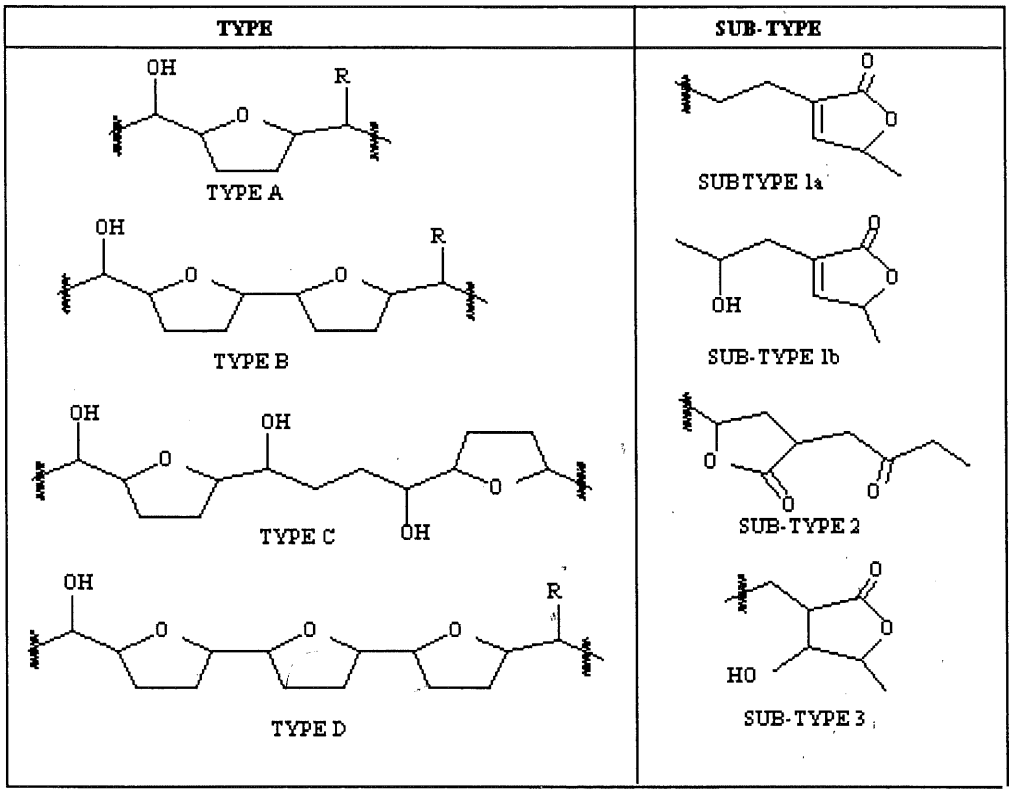

R = OH or H

Acetogenins from Annonaceae are a series of C-35/C-37 natural products of polyketide origin derived from fatty acids. Their structures are characterized by a long alkyl chain bearing a terminal methyl unsarurated γ-lactone (sometimes rearranged to a propanone γ-lactone), one, two or three tetrahydrofuran rings, several oxygen-nated substituents (hydroxyl, acetoxyl, ketone) and in some cases double bond(s) and or epoxy group(s). To date, they have been isolated only from the Annonaceae.

Annonaceous acetogenins exhibit a broad range of potent biological activities, especially antitumoral, cytotoxic, antiparasitic and pesticidal properties [4]. Due to the biological interest of these compounds, a lot of research have been undertaken; isolation, synthesis and biological studies.

In 1990, a review on acetogenins covered thirty one compounds belonging to this series [5]. Since then, more than one hundred and fifty new ones have been described. Acetogenins can be classified in four main groups A, B, C and D according to the number and arrangement of the tetrahydrofuran rings (Scheme 1):

- Type A acetogenins, with one tetrahydrofuran ring,
- Type B acetogenins, with two adjacent tetrahydrofuran rings.
- Type C acetogenins, with two tetrahydrofuran rings separated by a four carbon chain. Interestingly, in this type, one of the tetrahydrofuran ring possesses only one adjacent hydroxyl unlike the two other classes of acetogenin.
- Type D, with three adjacent tetrahydrofurans.




**Scheme 1**

These four main classes can be subdivided in three subtypes according to the lactone. Subtype 1, the most common, possesses an unsaturated γ-lactone, according to the presence or absence of a hydroxyl ß to the lactone. The sub-type can be divided into subtype 1a and 1b. That is justified by the special reactivity of the subtype 1b which, by an easy trans-lactonization, affords a saturated propanone-γ-lactone corresponding to the subtype 2.

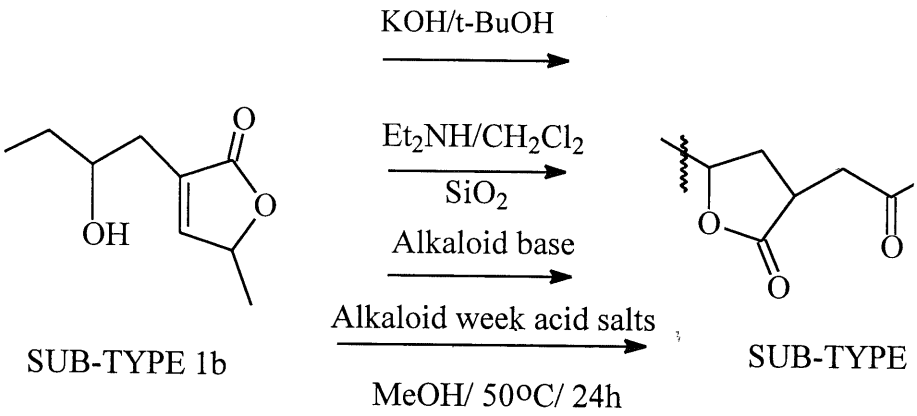
Subtype 2 or "isoacetogenins" is characterized by an acetonylbuty-rolactone. The acetogenins belonging to this subtype are actually artifacts formed during extraction or purfication processes as we have demonstrated [6,7]. Subtype 3 is characterized by the hydroxylation of the saturated lactone ring.

The fifth type is not typical of Annonaceous acetogenin since it does not possess any tetrahydrofuran ring but only one terminal unsaturated γ-lactone ring. This type is constituted

by products which are characterized by the presence along the chain of hydroxyl group, double bonds(s) and/or epoxy group(s) instead of the tetrahydrofuran. These products are "precursors" of the classical acetogenins. Isolation of acetogenins become easier with experience. Solvent extraction, solvent partition and chromatographies are used, guided either by bioassay or by TLC.

The TLC plates are generally stained by Kedde's reagent which is characteristic of an unsaturated γ-lactone. Sulfomolybdic reagent can also be used. Bioassayguided fractionation using lethality to brine shrimp larvae (BST) [8], appeared to be very useful owing to its rapidity, its low cost and its good correlation with the antitumor activity; both methods are complementary and are simultaneously used.

Some care must be taken during extraction, and also during isolation process to avoid the occurrence of artifacts. Infact, the acetogenins of subtype 1b are easily exposed to translactonization [6] (Scheme 2). It is obtained by subjecting an acetogenin possessing a hydroxyl at the C-4 to potash in butanol.




**Scheme 2**

But this reaction appears with a weak base such as diethylamine and also, more slowly, in presence of alkaloids, either as bases or as weak acid salts, this reaction being faster if the solution is heated. We have also demonstrated that the translactonization occurred in neutral or acidic medium by heating a methanolic solution. The process often requires ethanol or methanol for extraction or liquid liquid partition. On the other hand, Annonaceae often contain alkaloids which can favour translactonization due to their basicity. So it is recommended to avoid a long or important heating of the alcoholic solutions and any alkaline medium during extraction and isolation.

Isolation of acetogenins can be performed by column or circular partition chromatography. Isolation of pure products is not very easy because acetogenins exist as complex mixture of compounds of similar polarities. So the purity of isolated acetogenins must be controlled by High Performance Liquid Chromatography since it is the best method to ensure purity. Indeed, a one spot product on thin layer chromatography is sometimes a mixture of several compounds detected by HPLC.

This method allows to check the purity of isolated compounds, and it can be used as well for an analytical purpose. In spite of the absence of a large chromophore, the UV detection at 210-220 nm was satisfactory for the acetogenins of subtype-1. But in the course of our research we have demonstracted that UV detection was not convenient for the acetogenins of subtype-2 and subtype-3 because of the lack of chromophore in their molecule. For these reasons we have developed more universal techniques of detection as refractometric detection and evaporative light scattering detection (ELSD) [9].

Several preparative separations based on analytical HPLC results, using UV, refractrometric or evapo-

rative light scattering detection, and normal or reverse stationary phases [10] have been carried out. We have demonstrated that the HPLC technique is a rapid and sensitive method for identification and separation of different acetogenins. The chromatographic behaviour of the acetogenins in reverse phase HPLC can generally be interpreted according to their substitution patterns. The higher is the number of oxygenated groups, namely OH, the shorter is the retention time. However, other factors such as the position and type of substituents can change the elution order.

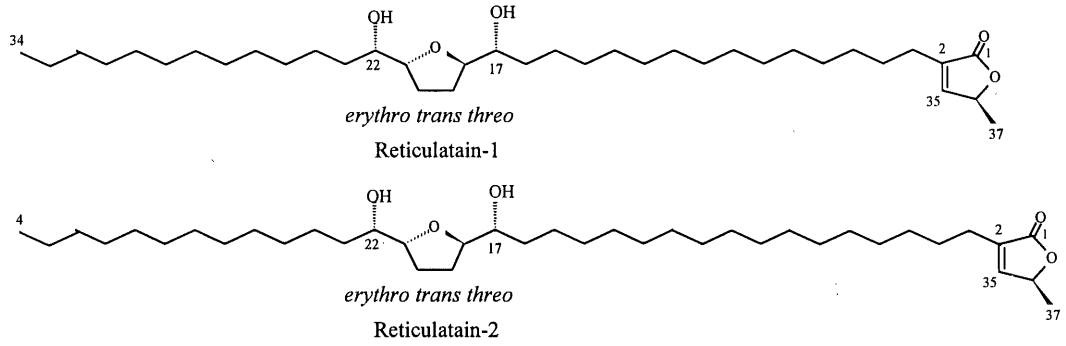



**Figure 1**

Liquid chromatography allows to separate several types of isomers, isomers of position such as reticulatain-1 and reticulatain-2 [11] (Figure 1), isomers differing by relative configuration such as rolliniastatin-1 and rolliniastatin-2 [12] (Figure 2) and even isomers differing by their absolute configuration at the level of the tetrahydrofuran pattern such as corossolone and 15, 16, 19, 20-epicorossolone [13] (Figure 3).

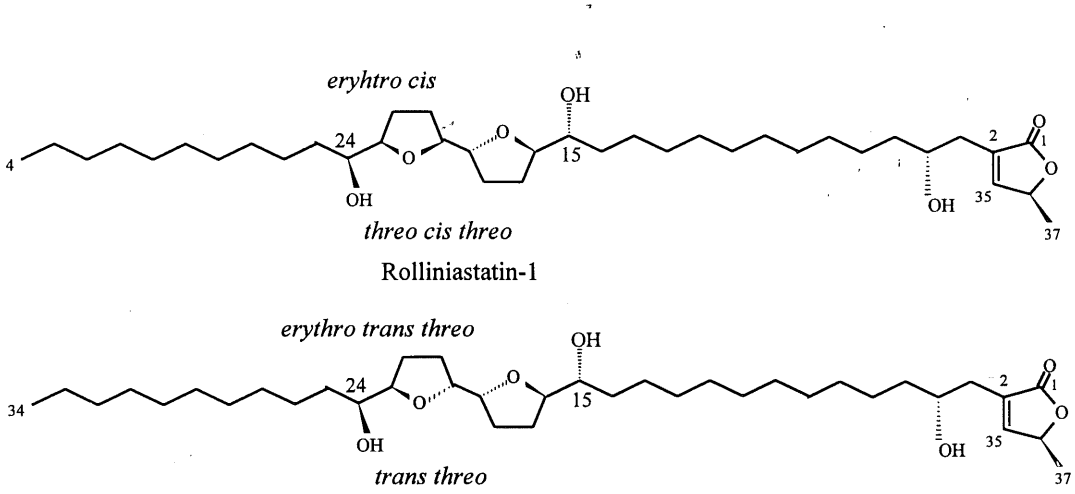



**Figure 2**

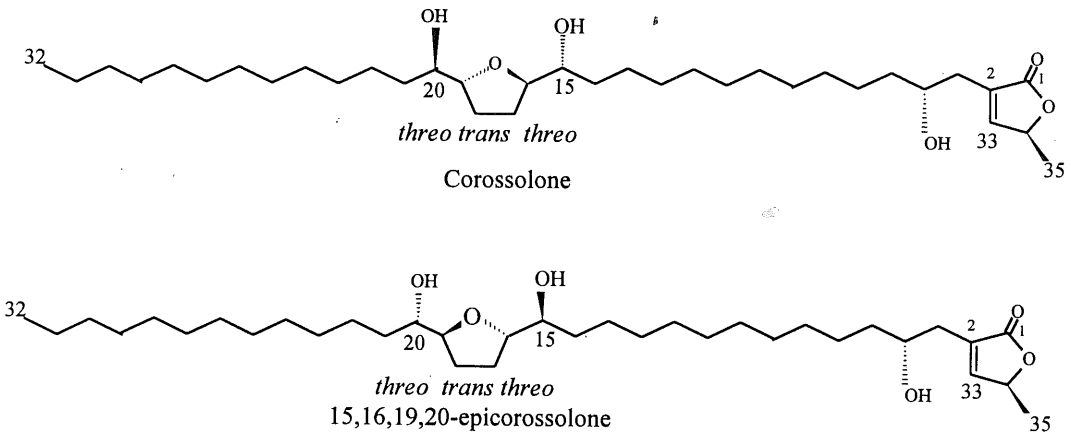



**Figure 3**

The successful separation of these diastereoisomeric compounds as well as the separation of cis and trans-isoacetogenins [14] shows the efficacy of the HPLC method for the analysis and separation of acetogenins from different Annonaceous plant extracts. Structural determination involves five stages:

- determination of the molecular weight;
- identification of the γ-lactone;
- characterization of the tetrahydrofuran pattern and determination of the nature and number of substituents along the carbon skeleton;
- placement of the tetrahydrofuran rings and substituents along the chain;
- determination of the stereochemistry.

Determination of the molecular weight requires chemical ionization mass spectrometry, fast atom bombardment mass spectrometry or fast atom bombardment in presence of lithium cations [15]. This last method, which is based on the property of acetogenins to form strong complexes with lithium, produces a simplification of the FAB mass spectrum as shown on the slide by suppression of peaks corresponding to sodium and potassium adducts.

Identification of the lactone type is easily performed by Kedde's reagent, IR and NMR. NMR also allows to characterize the tetrahydrofuran pattern and the $^{13}C$ NMR allows to correlate acetogenins to types A, B or C according to the number of carbon signals between 79 and 83 ppm. Nature and number of substituents along the carbon skeleton are demonstrated by NMR and preparation of derivatives, particularly acetylated and silylated derivatives [4].

Placement of the tetrahydrofuran ring(s) and substituents along the hydrocarbon chain is performed by careful analysis of the chemical ionization mass spectrum of acetogenins and their acetylated and trimethylsilylated derivatives.

The comparative analysis of mass spectra of the acetogenin of subtype-1 and those of their dihydroderivative obtained by catalytic hydrogenation can help to identify the fragments and determine the placement of substituents with certainty [16]. Moreover, based on the property of acetogenins to form complexes with lithium, we have shown that the placement may be also easily deduced, without any derivatization, from a careful examination of the B/E linked mass spectra obtained from $(M+Li)^+$ species generated by FAB [15, 17]. Indeed, the FAB mass spectrum recorded in presence of $Li^+$ cations, in addition of the molecular weight, yields unambiguously structurally significant ions. The B/E linked scan spectrum of theses significant ions allows to fix the position of the tetrahydrofuran and substituents.

Determination of the stereochemistry at the level of the level of the tetrahydrofuran ring and adjacent hydroxyls is now easily performed by comparison of the $^1H$ and $^{13}C$ NMR spectral data with those of synthetic models allowing to fix threo or erythro configurations [4].

In addition, COSY, relayed COSY and NOESY spectra studies are of great interest. The determination of the relative stereochemistry of the double bonds is easy by examination of the coupling constant. For the diols, examination of the NMR spectra of acetonides allow to fix threo or erythro configuration according to the proton values of methyl. If the configuration of the vicinal diol is *threo*, the two acetonyl proton Me signals will appear together as a six proton singlet; and if the configuration is *erythro*, the acetonyl Me proton signals will appear as two separated three proton singlets [18].

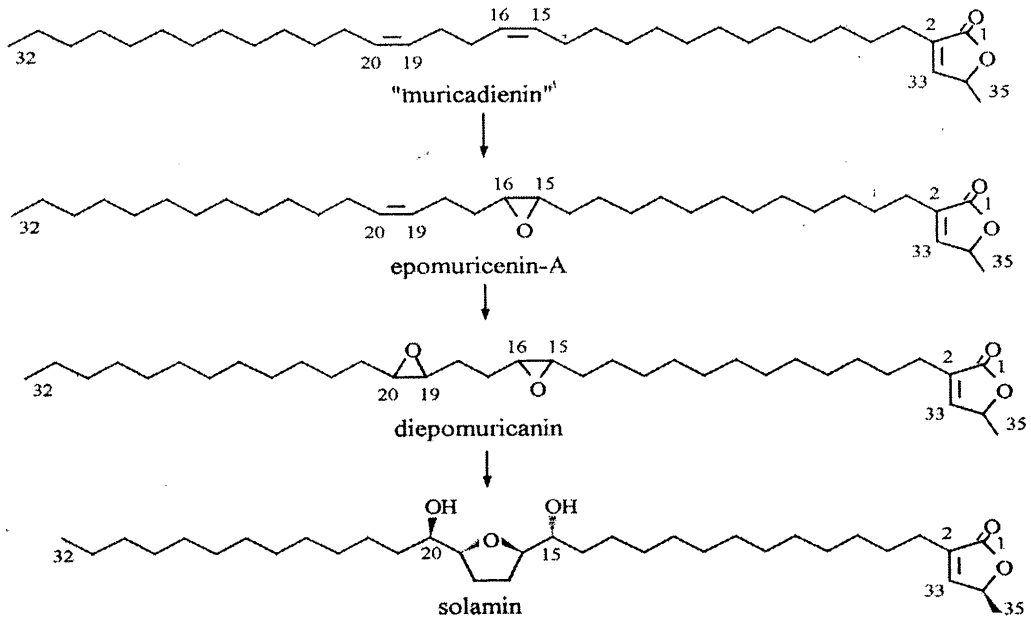
The preparation of formaldehyde acetal derivatives have also allowed to fix the relative configuration from the know configuration of the concerned hydroxyl. Acetogenins possessing 1, 2-, 1, 4- or 1, 5-diols have been derivatized through formaldehyde acetal in the presence of chlorotrimethylsilane ($Me_3SiCl$) and dimethylsulfoxide ($Me_2SO$) in excess [19].

This procedure affects only the diols without racemization of stereogenic centers. Observation of the signal corresponding to the acetalic methylene in $^1H$ NMR, allows the establishment of the relative stereo-chemical relationship of the ring formed. Indeed, in the case of a cis relationship, the two acetalic protons appear at 5.47 and 5.16 ppm, whereas for compounds of trans configuration, a singlet appears at 5.30 ppm.

For the determination of the absolute configuration, methods have been described consisting observations at high field of the 1H-NMR and 19F-NMR of Mosher esters [20]. This method is delicate; it needs a 500 MHz NMR spectrometer and allows to only fix the absolute configuration of the hydroxyls flanking the THF. However, owing to the association of relative determination of diols seen before, absolute configurations can be deduced.

Although no experimental work on the biosynthesis of Annonaceous acetogenins has been performed, hypothesis was proposed soon after the isolation of the first annonaceous acetogenin, uvaricin. At present, the hyphothesis are confirmed by the isolated of precursors and biomimetic hemisynthesis.

The lactone ring can be derived from fatty acid 2-monoglyceride. Some mono-acyl-2-glycerides have been isolated from *Annona senegalensis* seeds. The acyl part consists of palmitic, oleic and linoleic acids. Till now, no fatty acid made up of 32 or 34 carbons has been isolated among the fatty acids of Annonaceae [21].




Scheme 3

Tetrahydrofuran pattern aries from 1,5-dienes for acetogenins of type A and 1,5,9-trienes for acetogenins of type B. The biosynthesis involves the complete oxidation of double bonds and subsequent ring expansion of the oxirane thus derived from a diene or a triene. Reaction is a propagating one which is terminated when an alkoxide ion encounters a "defect" unit, in other words a separation of oxiranes by other than two carbon atoms. The limit can be an hydroxyl. That explains the existence of tetrahydrofuran mono-α-hydroxylated as in acetogenins from type C.

So, from the seeds of *Annona muricata* we have isolated epomuricenin A and diepomuricanin, in which localization of double bond and epoxy group are such that they can lead chemically to solamin which exists in the same plant. The hydpothésis that epomuricenin A could be the precursor of diepomuricanin was evidenced by epoxidation of its double bond which led to diepomuricanin [22]. Thus epomuricenin A and diepomuricanin emerge as possible links in the biogenetic pathway of monotetrahydrofuran acetogenins from polyunsaturated fatty acid derivatives dienic compounds such as the hypothetical muricadienin, which represent a missing link (Scheme 3).

There is now a lot of precursors isolated supporting this hypothesis. Recently we have isolated the first diene acetogenin, coriadienin [23] (Figure 4)

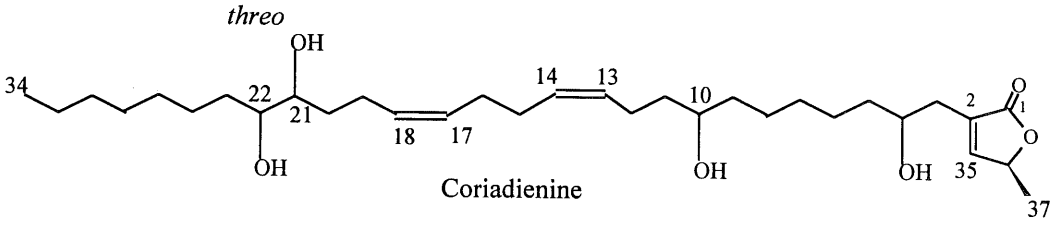



**Figure 4**

Since a few years total chiral synthesis of acetogenins from Annonaceae have been described [24]. The general approaches are based on a convergent strategy which consists of preparing both the tetrahydrofuran part and the lactonic moiety in a stereocontrolled way and finally the crosscoupling of the two synthons. For the tetrahydrofuran part two different approaches have been chosen: either starting from a chiral compounds such as an α- aminoacid or L-tartaric acid, or to apply an asymmetric catalyses reaction such as Sharpless asymmetric epoxidation or Sharpless asymmetric dihydroxylation.

The lactone moiety is prepared either from L-glutamic acid, from L-lactic acid, from (S)-malic acid or from (S)-propylene oxide. Acetogenins present a broad range of biological activities [4]. The first isolated acetogenin, uvaricin, was claimed as a "new antitumor agent". As a matter of fact, it presents a rather important *in vivo* cytotoxic activity against lymphocytic leukemia cells. It is now evident that all the acetogenins possess, to a vatiable degree, cytotoxic activity. This toxicity appears at concentration between $10^{-1}$ and $10^{-12}$ µg/ml depending on the acetogenin and the cell line.

Table 1 lists monotetrahydrofuranic acetogenins from *Annona muricata* which possess the same relative stereochemistry in the tetrahydrofuranic pattern (threo-trans-threo) [25].

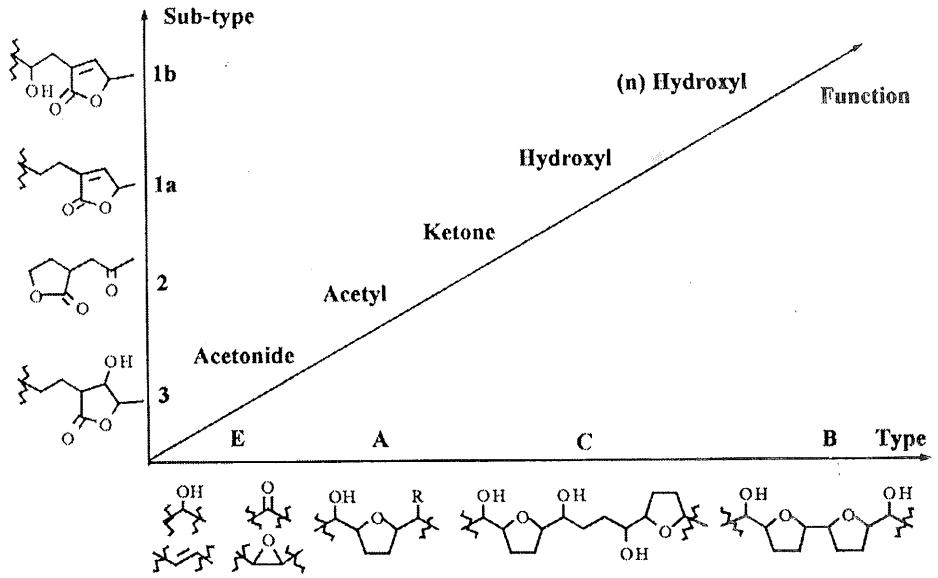
**Table 1.** Cytotoxicity of acetogenins isolated from Annona muricata

| Acetogenins | KB | VERO |
|---|---|---|
| annonacin | $10^{-4}$ | $10^{-2}$ |
| annonacinone | $10^{-2}$ | $10^{-1}$ |
| murisolin | $10^{-3}$ | $10^{-1}$ |
| corossolin | $3x10^{-3}$ | $3x10^{-2}$ |
| solamin | $3x10^{-1}$ | 1 |
| vincaleucoblastin* | $1x10^{-3}$ | >3 |

*reference

It should be stressed that activities are more pronounced for alcohol than for ketone derivatives, as shown by the couples annonacin/annonacinone and corossolin/corossolone. The presence of a hydroxyl on the chain seems to be important.

Corossolin has an activity at a lower concentration than murisolin, with one hydroxyl group at C-4, and annonacin, with two hydroxy groups at C-4 and C-10, has a stronger activity.




Scheme 4 Structure-cytotoxicity relationships

Acetylation of the hydroxy groups reduces the cytotoxic activity. It is also noteworthy that the lactone ring has an effect on the activity since its orupture strongly decreases activity, but the reduction of the double bond has very little influence. For the bistetrahydrofuran acetogenins, the same structure/activity relationship is observed. It seems that for the same level of hydroxylation, the bistetrahydrofuran derivatives are more cytotoxic than their monotetrahydrofuran counterparts.

A structure/cytotoxicity relationship compiled from various studies is presented in Scheme 4. It is difficult to draw conclusions on the significance of the absolute configuration of the various different asymmetric carbons, since this has not so far been determined for any acetogenin. Relative stereochemistry, at least, is clearly important: for instance, the described activity for asimicin and isomers, is from $10^{-3}$ to $10^{-5}$ while bullatacin has been claimed an activity at $10^{-13}$ with the relative configuration erythro-trans-threo-trans-threo.

In addition to assays on VERO and KB cells, studies of acetogenin activity have been carried out on cell line panels of human tumors such as colon, breast, pancreas, prostate, kidney, melanoma, ovary, etc... NCI has experimented various representative acetogenins on sixty human tumors cell panel and the average ED50 growth inhibition ranged from 1 to 10 mM [26].

Some acetogenins exhibit an antiparasitic activity. Uleicins from *Rollinia ulei* possess significant *in vitro* activity against *Leishmania donovani* with a good therapeutic index [27]. Similarly, acetogenins isolated from *Annona muricata* and from *Annona cherimolia* have a filaricidal activity against *Molinemma dessetae* [28]. Antimicrobial activity has also been observed. Beside these activities, pesticidal activity has been described, particularly for a lot of acetogenins. It is notable that, in some countries of South America, ground barks or seeds of some species of Annonaceae are spread on soils as pesticides.

Concerning the mechanism of action of these compounds, several hypothesis have been proposed. Some of them can be in relation with the capability of acetogenins to chelate monovalent or bivalent ions such as potassium and calcium and to act through a mechanism similar to that of antibiotics ionophore [29]. It has also been shown that acetogenins inhibit motochondrial electron transport through the NADH-ubiquinone oxidoreductase (respiratory complex I) and consequently inhibit the energy conserving function [30].

To conclude, acetogenins belong to a new class of pharmacologically active products. They have become the focus of an increasing attention from numerous research groups in recent years, owing to their exciting chemistry and also their wide spectrum of biological activities particularly their antitumor activity. A great progress has been made in the chemical part, extraction, structural determination and stereo-selective synthesis; a lot still remains to be done in the fields of biochemical pharmacology, and understanding of the mechanism of action.

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