Comparative SAR Evaluations of Annonaceous Acetogenins for Pesticidal Activity

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Abstract:

The activities of 44 Annonaceous acetogenins, which were originally isolated by monitoring plant fractionations with the brine shrimp lethality test (BST), were evaluated in the yellow fever mosquito larvae microtiter plate (YFM) assay. The results clearly demonstrate that most acetogenins have pesticidal properties. The structure–activity relationships indicate that the compounds bearing adjacent bis-THF (tetrahydrofuran) rings with three hydroxyl groups are the most potent. Bullatacin (**1**) and trilobin (**7**) gave the best activities against YFM with LC_{50} values of 0·10 and 0·67 mg litre^{-1}, respectively. Compounds showing LC_{50} values below 1·0 mg litre^{-1} in this assay are usually considered significant as new lead candidates for pesticidal development. In the BST, the corresponding LC_{50} values were $1·6×10^{-3}$ (**1**) and $9·7×10^{-3}$ (**7**) mg litre^{-1}. This is the first report of pesticidal structure–activity relationships for a series of Annonaceous acetogenins which are known to act, at least in part, as potent inhibitors of mitochondrial NADH: ubiquinone oxidoreductase.

Keywords: Annonaceous acetogenins | biopesticide | structure–activity relationship | brine shrimp lethality test | yellow fever mosquito larvae microtiter plate assay | plant extracts | mitochondrial enzyme inhibitors

Article:

1 INTRODUCTION

The Annonaceous acetogenins are naturally occurring long-chain fatty acid derivatives possessing unique structures and powerful antitumor and pesticidal activities. They are only found in the plant family, Annonaceae. In India, preparations from seeds, leaves, bark or fruit of *Annona muricata* L., *A. reticulata* L. and *A. squamosa* L. are widely employed by local people for emetic, astringent, insecticidal, pesticidal, anthelminthic and antidysenteric purposes.[1] The report of the first acetogenin, uvaricin, in 1982,[2] stimulated interest in the discovery of additional new bioactive compounds of this type. To date, more than 220 acetogenins have been isolated from 26 Annonaceous species by bioassay-directed fractionation and phytochemical isolation methods.[3] These Annonaceous acetogenins have exhibited a diversity of bioactivities including antitumor, pesticidal, insect antifeedant, antimalarial, T-cell suppressant, antiparasitic and antimicrobial properties.[4-9]

The chemical structures of the acetogenins are considered to be derived from C-32 or C-34 long-chain fatty acids which are combined with a 2-propanol unit at C-2 to form an α,β-unsaturated γ-lactone ring. In the biogenesis, double bonds along the fatty acid chain seem to epoxidize and cyclize to form tetrahydrofuran (THF) rings. According to the number and positions of the THF rings, the linear acetogenins are classified in mono-THF, adjacent bis-THF, non-adjacent bis-THF and tri-THF ring systems. One compound (mucocin, **21**) has recently been found which has a tetrahydropyran (THP) ring as well as a non-adjacent THF ring.[3] The sources, biogenesis, isolation, chemistry, synthesis and biological activities of Annonaceous acetogenins have been extensively reviewed.[3,10-13]

The remarkable pesticidal, insecticidal and antifeedant properties of the Annonaceous acetogenins have been reported[14-16] and patented.[5-7] A standardized extract of *Asimina triloba* Dunal, which is rich in acetogenins, shows strong pesticidal activities. Asimicin (**11**), a major biologically active component isolated from this species, demonstrated promising activities against Mexican been beetles, *Epilachna varvestis* Muls., melon aphids, *Aphis gossypii* Glov., mosquito larvae, *Aedes aegypti* L., the nematode, *Caenorhabditis elegans* Maupas, blowfly larvae, *Calliphora vicina* R. & D. and striped cucumber beetles, *Acalymma vittata* F.[16] The pesticidal effect of the acetogenins is exerted, at least in part, via inhibition of complex I (NADH: ubiquinone oxidoreductase) in mitochondrial electron transport systems; this inhibition results in the deprivation of ATP at cellular levels.[16-18]

Examination of a series of structurally diverse Annonaceous acetogenins in an assay with yellow fever mosquito (*Ae. aegypti*) larvae, enables us to evaluate the relative potencies of their bioactivities as well as their pesticidal structure-activity relationships. Acetogenins with pesticidal properties used in this study possess several common structural features : they contain one or two THF (or THP) rings with one or two flanking hydroxyl groups; the bis-THF rings can be either adjacent or non-adjacent. A γ-lactone ring is at one terminal, and other functional groups include hydroxyls, acetoxyls and double bonds (Figs 1-4).

2 EXPERIMENTAL

2.1 Isolation of acetogenins

All the acetogenins used in this study were isolated from either *Annona muricata* L., *Annona bullata* Rich., *Asimina longifolia* Kral, *Asimina triloba* Dunal, *Goniothalamus giganteus* Hook. f. and Thomas, or *Rollinia mucosa* Baill. by our research group. The isolations were bioassay-directed using the brine shrimp (*Artemia salina* L.) lethality test (BST).[19,20] The plant extracts contain complex mixtures of acetogenins with some compounds present in very small concentrations ; thus, repeated chromatographic methods are required to separate their mixtures. In our laboratory, Alkofahi et al.[14] have reported a general scheme for the isolation which involves extraction with ethanol of the selected plant materials (usually bark, leaves or seeds) to obtain the residue of the ethanol extract (F001). F001 is then subjected to partitioning between chloroform (or dichloromethane) and water to give the water-soluble (F002), chloroform-soluble (F003) and insoluble interface residues (F004). F003 is further partitioned between hexane and 90% aqueous methanol to produce methanol-soluble (F005) and hexane-soluble (F006) residues. The pure acetogenins are obtained from F005 by repeated chromatography over open columns (silica gel), chromatotrons and/or HPLC (normal and reversed phases). The BST is routinely employed to monitor the extraction and isolation of the bioactive compounds, and the results correlate well with a series of pesticidal tests.[14] The LC_{50} values in the BST for the acetogenins are listed in Table 1 for the purpose of comparison with the results of the YFM test. The LC_{50} values of rote-none are included as a positive control. The purities and characterizations of each isolated acetogenin were evaluated by TLC, HPLC, [^{1}H]NMR and [^{13}C]NMR, and their sources and appropriate reference publications are cited in our published reviews.[3,10-12]

2.2 Yellow fever mosquito microtiter plate assay

The yellow fever mosquito larvae microtiter plate assay (YFM)[21] was used to determine the relative pesticidal activities of the series of acetogenins (Table 1). The yellow fever mosquito (*Ae. aegypti*) eggs were provided by Dr Steve Sackett (New Orleans Mosquito Control Board, New Orleans, Louisiana 70126). The eggs were placed into 200-300 ml of water and were allowed to hatch overnight. The larvae were then transferred to a 400-ml beaker containing bovine liver powder (ICN Biochemicals) at a concentration of 2-4 g litre^{-1} and allowed to develop for four days. Harvested living larvae were transferred with a 10-ml plastic transfer pipette into a 20-ml scintillation vial filled with MES (2-[*N*-morpholino]ethanesulfonic acid,) buffer (5 mM, pH 6·5; 10 ml) ; the larvae were then ready for the following experiment. A Vaccupette/96 was used to fill 96-well flat bottom assay plates with 240 μl of 5 mM MES per well. A single larva was added per well by using a Model edp microliter repipettor (Rainin Instrument), and the test acetogenins in methanol (5 μl) were subsequently introduced to each well. Rotenone was used as a positive control and a 20 ml litre^{-1} methanol solution was run as a negative control in each experiment. Dilutions (1 : 10) of test compounds in methanol were prepared starting at 500 mg litre^{-1} or 50 mg litre^{-1}, depending upon the available quantities of each sample. Two compounds per plate and eight replicates per dose were used. After adding the solutions of the test compounds, the plates were covered and incubated in the dark at 25-28°C for four days. The plate was then scored for dead larvae. LC values and 95% confidence 50 intervals in mg litre^{-1} were calculated using a Finney Probit analysis computer program,[20] and the relative activities of each acetogenin were subsequently evaluated (Table 1).

3 RESULTS AND DISCUSSION

Based on the results of the YFM assay, as shown in Table 1, several general conclusions about the pesticidal structure-activity relationships (SAR) of Annonaceous acetogenins can be drawn: (i) most acetogenins tested in this study are toxic to the yellow fever mosquito larvae at concentrations of less than 50 mg litre^{-1}, (ii) the adjacent or non-adjacent bis-THF ring acetogenins show higher potencies of activity than the mono-THF ring compounds which possess the same number of hydroxyl groups; and (iii) the potency of activity is related to the number and the positions of the hydroxyl groups and the positions of the THF rings.

Bullatacin (**1**) and trilobin (**7**) exhibited activities superior to those of the other acetogenins. The high potencies of bullatacin and trilobin are not restricted to the YFM assay. They are also found to be two of the most powerful acetogenins in human solid tumor cell cytotoxicity and rat liver mitochondrial inhibition assays,[12,22-25] and they are among the four most potent acetogenins in the BST (Table 1). The two compounds have similar levels of bioactive potencies. A positional shift of the third hydroxyl group, as well as the stereoconfiguration of the bis-THF ring system, seems to influence the potencies, e.g. in the cases of motrilin (**3**), trilobacin (**8**), asiminacin (**10**) and asimicin (**11**).

The importance of the polarities of the molecules in affecting the biological activities of acetogenins has been recognized for some time. The large potency difference (a factor of 10) between bullatacin and bullatetrocin (**2**), 12-hydroxy bullatacin A (**4**), 12-hydroxy bullatacin B (**5**) and rollitacin (**6**), which all possess four hydroxyl groups, indicates the strong influence of the number of hydroxyl groups. The extra hydroxyl group in these four-hydroxylated bullatacin type acetogenins increases the polarity of the molecules but decreases their bioactivities. A similar situation is observed between asimicin and 10-hydroxy asimicin (**12**) in which asimicin, with one less hydroxyl, has enhanced potency. It appears that, to obtain optimum activities, three hydroxyl groups are required in adjacent bis-THF acetogenin molecules.

We also note that the position of the THF rings in the acetogenin molecules has a remarkable effect on the specific structural requirements for activity, i.e. a suitable chain length between the THF ring and γ-lactone ring is required. Bullatacin (**1**), trilobin (**7**), asiminacin (**10**), asimicin (**11**), trilobacin (**8**) and motrilin (**3**) all have the THF rings located at C-15 to C-24 relative to the terminal γ-lactone ring, and all show highly potent bioactivities against yellow fever mosquito larvae, ranging from 0·10 to 5·0 mg litre^{-1}. Among the several acetogenins of the asimicin type, asiminacin (**10**) and asimicin (**11**) are active compounds in the YFM assay with LC$_{50}$ values of 1·6 and 2·7 mg litre^{-1}, respectively. Longimicins B (**14**), C (**15**) and D (**13**) are structurally different from asiminacin and asimicin in that their THF ring systems are shifted along the chain toward the clactone. Longimicin D (13, THF rings at C-13 to C-22 and the third hydroxyl at C-10 instead of C-4) and longimicin B (14, THF rings at C-11 to C-20) show a decreasing trend of activities, with LC$_{50}$ values of 11·5 and 27·3 mg litre^{-1}, respectively ; while longimicin C (**15**), with the ring position further shifted to C-9 to C-18, has its activity further reduced to LC$_{50}$ 65·7 mg litre^{-1}.

The three non-adjacent THF ring acetogenins, *cis*- and *trans*-sylvaticin (**17** and **16**) and bullatalicin (**18**), exhibited comparable toxicity to the mosquito larvae, although they contain four hydroxyl groups. It is, therefore, suggested that a THF ring at C-20 might be important for maximal bioactivity. The recently discovered acetogenin, mucocin (**21**), with a tetrahydropyran

(THP) ring at C-20 and a THF ring at C-12, showed very good activity at LC_{50} 2·1 mg litre^{-1}. This is the first reported acetogenin with a THP ring.[26]

The mono-THF ring acetogenins, while less potent, still displayed similar SAR trends to those with the bis-THF ring systems. Both the hydroxyl numbers and the positions of the THF rings are important in determining their relative potencies. The most active mono-THF ring compounds tested in this study were those molecules with four hydroxyl groups and with the THF ring positioned at C-15 to C-20, such as in annonacin (**22**) and annonacin A (**23**). With the shifts of the THF ring to C-17, such as in rollinecins A (**27**, LC_{50} > 50 mg litre^{-1}) and B (**28**, LC_{50} > 50 mg litre^{-1}) or to C-13, and/or the loss of one of the four hydroxyl groups, significant decreases in the activities were observed, e.g. in longicoricin (**30**, LC_{50} 58·0 mg litre^{-1}) and in longifolicin (**31**, LC_{50} 249 mg litre^{-1}), respectively. However, a close structural relative of longifolicin is giganenin (**29**); giganenin exhibited activity 20-fold higher than that of longifolicin, suggesting that the double bond in giganenin plays an important role in biopotency.

The class of mono-THF ring acetogenins having one flanking hydroxyl group gives maximum activities with three or four hydroxyl groups, e.g., gigantetrocinone (**32**), gigantrionenin (**33**), gigantetroneninone (**35**), *cis*-gigantrionenin (**34**) and gigantetrocin A (**37**). Three five-hydroxyl (**40–42**) and two six-hydroxyl (**43** and **44**) substituted mono-THF acetogenins were found to be inactive at concentrations less than 50 mg litre^{-1}. When the THF ring was shifted from C-9 to C-11 (muricatetrocin C, **39**), activity against YFM but not BST was lost at concentrations of <50 mg litre^{-1}. Muricatetrocin B (**38**), a C-20 epimer of muricatetrocin C, exhibited significant activity over its isomer that could only be explained by stereoselectivity. A similar example is exhibited between the C-24 epimers, rollidecins A (**19**) and B (**20**), where A showed activity at LC_{50} 11·2 mg litre^{-1}, while no activity was observed at concentrations of <50 mg litre^{-1} in rollidecin B. Once again, although activity against YFM was lost in going from rollidecin A to B, activity in the BST was not lost but increased slightly in going from **19** to **20**.

4 CONCLUSIONS

The pesticidal properties of the Annonaceous acetogenins have been known for several years.[6] The pesticidal SARs indicate that, of all the acetogenins tested so far, the adjacent bis-THF ring molecules with three hydroxyl groups are the most potent. Bullatacin (**1**) and trilobin (**7**) are the strongest and give LC_{50} values against YFM of 0·10 and 0·67 mg litre^{-1}, respectively; while rotenone, as a positive control gave LC 1É2 mg 50 litre^{-1}. Usually, compounds showing LC_{50} values below 1·0 mg litre^{-1} in the YFM test are considered significant as new leads for pesticidal development.

Acetogenins show a tremendous potential for development as new natural pesticides due to their potent bioactivities, their known mechanism of action, their natural origin and their expected low oral toxicities in higher animals (they are emetic).[27] The use of natural acetogenin mixtures in plant extracts is predicted as an economical and practicable means for pest control. For example, two Annonaceous species, *Asimina triloba* Dunal (paw paw) and *Annona muricata* L. (sour sop, guanabana), are, respectively, abundant as fruit trees in temperate eastern North America and in the tropics worldwide. Both of these species have now yielded a variety of new acetogenins (over 30 acetogenins each), and their crude extracts exhibit potent pesticidal effects. These

extracts, which can be easily prepared, can be employed as safe, effective, economical and environmentally friendly pesticides with an emphasis on the home garden, ornamental, greenhouse and produce markets, pending regulatory approval.[27]

ACKNOWLEDGEMENTS

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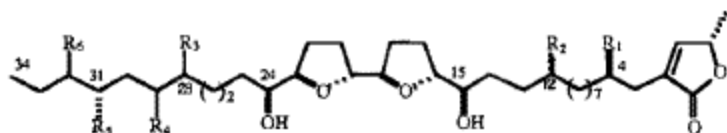
bullatacin: R_1=OH, R_2=R_3=R_4=R_5=R_6=H (**1**)
bullatetrocin: R_1=R_2=R_3=R_4=H, R_5=R_6=OH (**2**)
motrilin: R_1=R_2=R_3=R_5=R_6=H, R_4=OH, *S* (**3**)
12-hydroxy bullatacin A: R_1=OH, R_2=OH, *R*, R_3=R_4=R_5=R_6=H (**4**)
12-hydroxy bullatacin B: R_1=OH, R_2=OH, *S*, R_3=R_4=R_5=R_6=H (**5**)
rollitacin: R_1=R_2=R_5=R_6=H, R_3=R_4=OH (**6**)
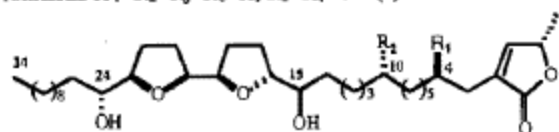
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trilobacin: R_1=OH, R_2=H (**8**)
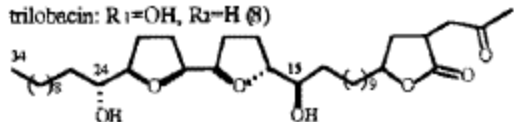
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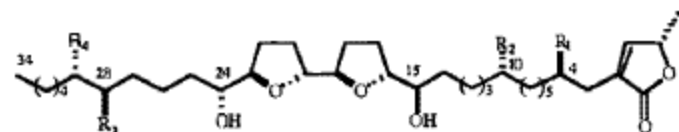
asiminacin: R_1=R_2=H, R_3=OH, R_4=H (**10**)
asimicin: R_1=OH, R_2=H, R_3=R_4=H (**11**)
10-hydroxy asimicin: R_1=OH, R_2=OH, R_3=R_4=H (**12**)
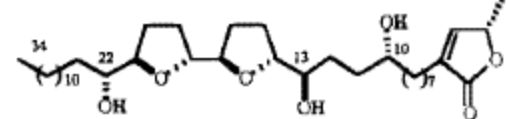
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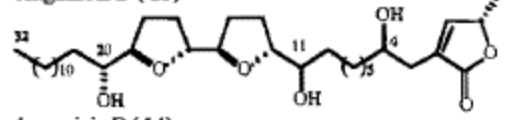
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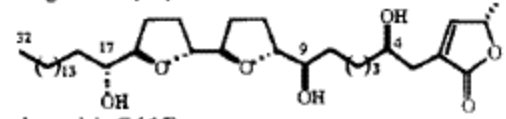
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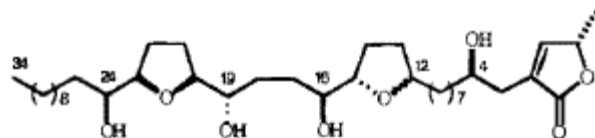
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cis-**sylvaticin 12S (17)**
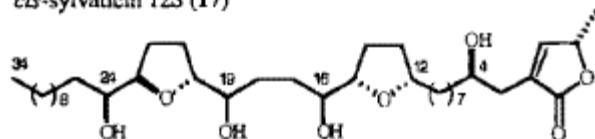
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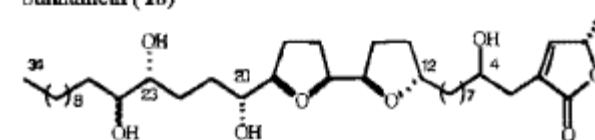
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rollidecin B: 24 S (20)

mucocin (21)
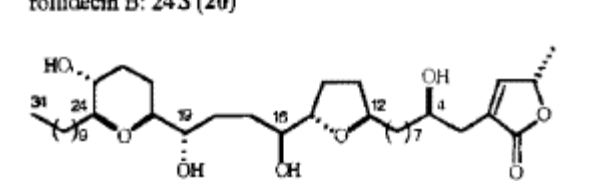
Fig. 1. Bis-THF ring acetogenins.

Fig. 2. Non-adjacent bis-THF ring, adjacent bis-THF ring with one flanking hydroxyl and non-adjacent tetrahydropyran and mono-THF ring acetogenins.
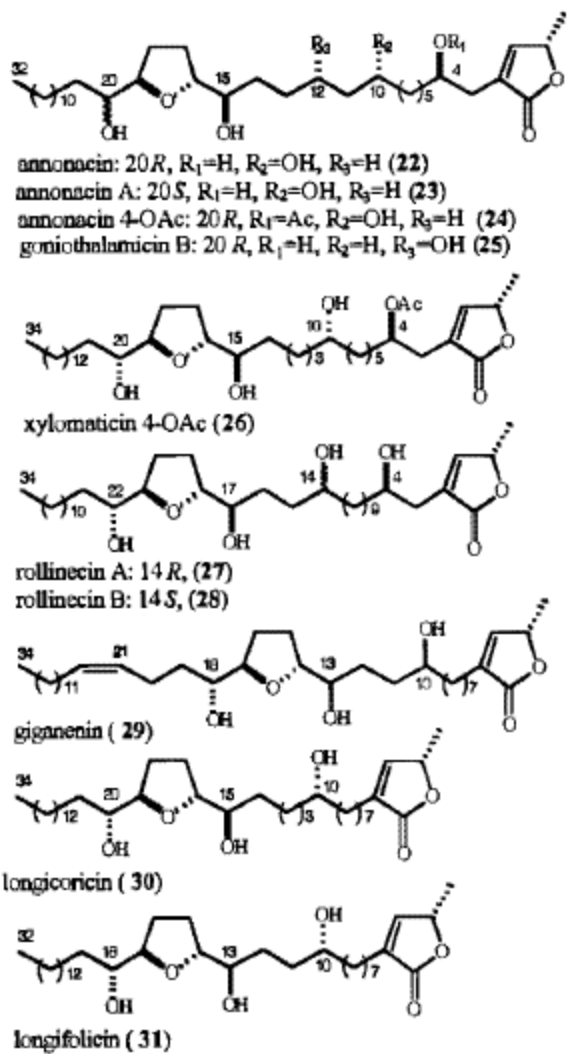
Fig. 3. Mono-THF ring acetogenins with two flanking hydroxyls.

annonacin: 20*R*, R₁=H, R₂=OH, R₃=H (**22**)
annonacin A: 20*S*, R₁=H, R₂=OH, R₃=H (**23**)
annonacin 4-OAc: 20*R*, R₁=Ac, R₂=OH, R₃=H (**24**)
goniothalamicin B: 20 *R*, R₁=H, R₂=H, R₃=OH (**25**)

xylomaticin 4-OAc (**26**)

rollinecin A: 14 *R*, (**27**)
rollinecin B: 14 *S*, (**28**)

giganenin (**29**)

longicoricin (**30**)

longifolicin (**31**)
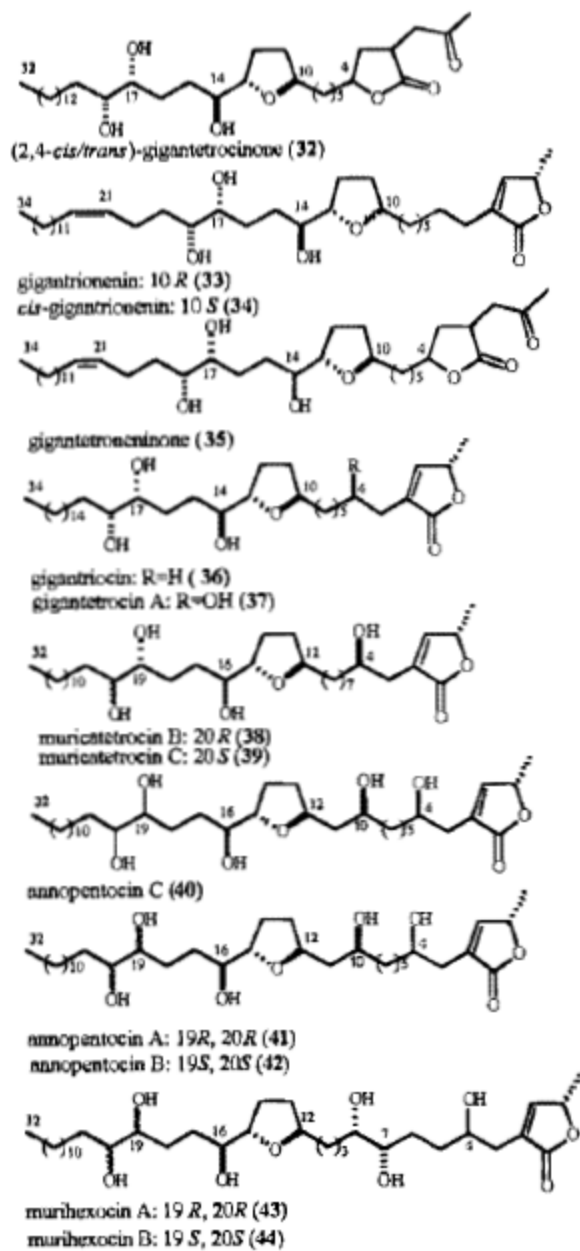
Fig. 4. Mono-THF ring acetogenins with one flanking hydroxyl.

(2,4-*cis/trans*)-gigantetrocinone (**32**)

gigantrionenin: 10 *R* (**33**)
cis-gigantrionenin: 10 *S* (**34**)

gigantetroneninone (**35**)

gigantriocin: R=H (**36**)
gigantetrocin A: R=OH (**37**)

muricatetrocin B: 20 *R* (**38**)
muricatetrocin C: 20 *S* (**39**)

annopentocin C (**40**)

annopentocin A: 19*R*, 20*R* (**41**)
annopentocin B: 19*S*, 20*S* (**42**)

murihexocin A: 19 *R*, 20*R* (**43**)
murihexocin B: 19 *S*, 20*S* (**44**)

Table 1. Results of Brine Shrimp Lethality (BST) and Yellow Fever Mosquito (YFM) Larvae Tests for Annonaceous Acetogenins

Compounds	BST activities (LC$_{50}$ mg litre^{-1})[a]	YFM activities (LC$_{50}$ mg litre^{-1})[a]
Bullatacin (**1**)	$1 \cdot 6 \times 10^{-3}$ ($0 \cdot 8 \times 10^{-3}$–$12 \cdot 4 \times 10^{-2}$)	$1 \cdot 0 \times 10^{-1}$ ($2 \cdot 0 \times 10^{-2}$–$3 \cdot 9 \times 10^{-1}$)
Trilobin (**7**)	$9 \cdot 7 \times 10^{-3}$ ($5 \cdot 3 \times 10^{-3}$–$1 \cdot 6 \times 10^{-2}$)	$6 \cdot 7 \times 10^{-1}$ ($3 \cdot 7 \times 10^{-2}$–$2 \cdot 1 \times 10^{-1}$)
Trilobacin (**8**)	$8 \cdot 7 \times 10^{-3}$ ($5 \cdot 2 \times 10^{-3}$–$14 \cdot 4 \times 10^{-3}$)	$1 \cdot 6$ ($5 \cdot 0 \times 10^{-1}$–$5 \cdot 0$)
Asiminacin (**9**)	$5 \cdot 7 \times 10^{-3}$ ($3 \cdot 5 \times 10^{-3}$–$9 \cdot 0 \times 10^{-3}$)	$1 \cdot 6$ ($5 \cdot 0 \times 10^{-1}$–$5 \cdot 0$)
Asimicin (**11**)	$2 \cdot 6 \times 10^{-2}$ ($1 \cdot 5 \times 10^{-2}$–$5 \cdot 3 \times 10^{-2}$)	$2 \cdot 7$ ($8 \cdot 1 \times 10^{-1}$–$8 \cdot 7$)
Motrilin (**3**)	$1 \cdot 0 \times 10^{-2}$ ($0 \cdot 6 \times 10^{-3}$–$1 \cdot 9 \times 10^{-2}$)	$4 \cdot 7$ ($1 \cdot 6$–$14 \cdot 1$)
Rollitacin (**6**)		$4 \cdot 7$ ($1 \cdot 6$–$14 \cdot 1$)
Bullatetrocin (**2**)	$3 \cdot 1 \times 10^{-1}$ ($2 \cdot 0 \times 10^{-1}$–$4 \cdot 6 \times 10^{-1}$)	$6 \cdot 2$ ($1 \cdot 9$–$18 \cdot 9$)
12-OH Bullatacin A (**4**)		$8 \cdot 0$ ($2 \cdot 0$–$27 \cdot 7$)
12-OH Bullatacin B (**5**)		$8 \cdot 0$ ($2 \cdot 0$–$27 \cdot 7$)
Trilobacinone (**9**)	$3 \cdot 1 \times 10^{-1}$ ($1 \cdot 6 \times 10^{-1}$–$5 \cdot 3 \times 10^{-1}$)	$10 \cdot 4$ ($1 \cdot 4$–$28 \cdot 9$)
10-OH Asimicin (**12**)	$4 \cdot 3 \times 10^{-1}$ ($2 \cdot 8 \times 10^{-1}$–$6 \cdot 7 \times 10^{-1}$)	$15 \cdot 2$ ($4 \cdot 8$–$30 \cdot 9$)
Longimicin D (**10**)	$4 \cdot 6$ ($1 \cdot 3$–$9 \cdot 2$)	$11 \cdot 5$ ($3 \cdot 5$–$31 \cdot 5$)
Longimicin B (**14**)	$7 \cdot 3$ ($4 \cdot 0$–$15 \cdot 7$)	$27 \cdot 3 (8 \cdot 1$–$87 \cdot 3$)
Longimicin C (**15**)	$9 \cdot 4$ ($6 \cdot 6$–$18 \cdot 4$)	$65 \cdot 7$ ($18 \cdot 6$–$171 \cdot 8$)
Sylvaticin (**16**)	$8 \cdot 0 \times 10^{-1}$ ($0 \cdot 34$–$1 \cdot 7$)	$1 \cdot 6$ ($5 \cdot 0 \times 10^{-1}$–$5 \cdot 0$)
cis-Sylvaticin (**17**)	$1 \cdot 1$ ($6 \cdot 0 \times 10^{-1}$–$2 \cdot 7$)	$6 \cdot 0$ ($1 \cdot 7$–$23 \cdot 4$)
Bullatalicin (**18**)	$1 \cdot 5 \times 10^{-1}$ ($5 \cdot 1 \times 10^{-2}$–$2 \cdot 9 \times 10^{-1}$)	$9 \cdot 0$ ($2 \cdot 4$–$57 \cdot 1$)
Rollidecin A (**19**)	$4 \cdot 2 \times 10^{-1}$ ($2 \cdot 6 \times 10^{-1}$–$6 \cdot 7 \times 10^{-1}$)	$11 \cdot 2$ ($3 \cdot 5$–$31 \cdot 3$)
Rollidecin B (**20**)	$2 \cdot 8 \times 10^{-1}$ ($1 \cdot 4 \times 10^{-1}$–$4 \cdot 3 \times 10^{-1}$)	> 50
Mucosin (**21**)	$1 \cdot 8$ ($1 \cdot 0$–$1 \cdot 8$)	$2 \cdot 1$ ($6 \cdot 4 \times 10^{-1}$–$6 \cdot 8$)
Annonacin (**22**)	$3 \cdot 1 \times 10^{-1}$ ($1 \cdot 7$–$7 \cdot 4$)	$3 \cdot 0$ ($8 \cdot 0 \times 10^{-1}$–$6 \cdot 7$)
Xylomaticin 4-OAc (**26**)	$34 \cdot 1$ ($11 \cdot 8$–$53 \cdot 2$)	$6 \cdot 2$ ($1 \cdot 9$–$18 \cdot 9$)
Annonacin 4-OAc (**24**)	$21 \cdot 9$ ($15 \cdot 3$–$40 \cdot 1$)	$6 \cdot 2$ ($1 \cdot 9$–$18 \cdot 9$)
Giganenin (**29**)	$89 \cdot 0$ ($65 \cdot 0$–$101 \cdot 4$)	$7 \cdot 8$ ($2 \cdot 0$–$26 \cdot 1$)
Annonacin A (**23**)	$8 \cdot 3 \times 10^{-1}$ ($4 \cdot 8 \times 10^{-1}$–$1 \cdot 4$)	$10 \cdot 8$ ($1 \cdot 4$–$28 \cdot 9$)
Goniothalamicin B (**25**)		$13 \cdot 3$ ($3 \cdot 6$–$29 \cdot 7$)
Rollinecin A (**27**)	$3 \cdot 6 \times 10^{-1}$ ($5 \cdot 3 \times 10^{-2}$–$1 \cdot 5 \times 10^{-1}$)	> 50
Rollinecin B (**28**)	$1 \cdot 3 \times 10^{-1}$ ($6 \cdot 6 \times 10^{-2}$–$2 \cdot 2 \times 10^{-1}$)	> 50
Longifolicin (**31**)	$3 \cdot 5$ ($1 \cdot 8$–$6 \cdot 3$)	249 ($50 \cdot 3$–600)
Longicoricin (**30**)	$1 \cdot 6$ ($0 \cdot 3$–$2 \cdot 7$)	$58 \cdot 0$ ($12 \cdot 1$–$101 \cdot 6$)
Muricatetrocin B (**38**)	$1 \cdot 8$ ($8 \cdot 5 \times 10^{-1}$–$3 \cdot 8$)	$2 \cdot 2$ ($5 \cdot 7 \times 10^{-1}$–$7 \cdot 3$)
Gigantetrocinone (**32**)	$1 \cdot 3 \times 10^{-1}$ ($1 \cdot 0 \times 10^{-1}$–$2 \cdot 0 \times 10^{-1}$)	$2 \cdot 2$ ($5 \cdot 7 \times 10^{-1}$–$7 \cdot 3$)
Gigantrionenin (**33**)	$13 \cdot 9$ ($5 \cdot 3$–$31 \cdot 5$)	$4 \cdot 0$ ($1 \cdot 3$–$13 \cdot 2$)
Gigantetroneninone (**35**)	$1 \cdot 1 \times 10^{-1}$ ($1 \cdot 7 \times 10^{-2}$–$2 \cdot 3 \times 10^{-1}$)	$8 \cdot 3$ ($2 \cdot 8$–$14 \cdot 8$)
cis-Gigantrionenin (**34**)	$2 \cdot 5$ ($1 \cdot 3$–$4 \cdot 3$)	$8 \cdot 6$ ($2 \cdot 2$–$33 \cdot 7$)
Gigantetrocin A (**37**)	$6 \cdot 0 \times 10^{-1}$ ($3 \cdot 5 \times 10^{-1}$–$1 \cdot 9$)	$10 \cdot 4$ ($1 \cdot 4$–$28 \cdot 9$)
Gigantriocin (**36**)	$5 \cdot 6$ ($2 \cdot 0$–$10 \cdot 0$)	$18 \cdot 5$ ($4 \cdot 1$–$44 \cdot 9$)
Muricatetrocin C (**39**)	$7 \cdot 6 \times 10^{-1}$ ($2 \cdot 5 \times 10^{-1}$–$1 \cdot 4$)	> 50
Annopentocin A (**41**)	$8 \cdot 9$ ($4 \cdot 5$–$20 \cdot 5$)	> 50
Annopentocin B (**42**)	$11 \cdot 2$ ($6 \cdot 3$–$21 \cdot 3$)	> 50
Annopentocin C (**40**)	$13 \cdot 8$ ($5 \cdot 4$–$31 \cdot 2$)	> 50
Murihexocin A (**43**)	$28 \cdot 6$ ($15 \cdot 5$–$62 \cdot 1$)	> 50
Murihexocin B (**44**)	$33 \cdot 7$ ($18 \cdot 7$–$70 \cdot 8$)	> 50
Rotenone (positive control)	$4 \cdot 9 \times 10^{-2}$ ($2 \cdot 3 \times 10^{-2}$–$9 \cdot 3 \times 10^{-2}$)	$1 \cdot 2$ ($1 \cdot 2 \times 10^{-1}$–$4 \cdot 1$)

[a] 95% confidence intervals in parentheses