

バンレイシ(*Annona squamosa* L.)種子に含まれるバンレイシ科テトラヒドロフランアセトゲニン類の構造決定、生理活性

| | |
|-------|-------------|
| 誌名 | 農業環境技術研究所報告 |
| ISSN | 09119450 |
| 著者名 | 荒谷,博 |
| 発行元 | 農業環境技術研究所 |
| 巻/号 | 23号 |
| 掲載ページ | p. 77-149 |
| 発行年月 | 2004年3月 |

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



[Bull. Natl. Inst. Agro-Environ.
Sci., 23, 77-149 (2004)]

Studies on Annonaceous Tetrahydrofuranic Acetogenins from *Annona squamosa* L. Seeds

Hiroshi Araya *

(Received February 13, 2004)

Synopsis

The method for structure elucidation of annonaceous tetrahydrofuranic acetogenins, which have attracted much interest in these years because of potent various biological activities, were studied. In the course of the investigation, seven new acetogenins and sixteen known acetogenins were isolated from seeds of *Annona squamosa* L. (Annonaceae) collected in India. All of their structures were determined by spectroscopic methods. The planar structures of some acetogenins have been elucidated by newly developed method (amine method), namely, the application of precursor ion scanning in mass spectrometry. Thus, these compounds were converted into lactam derivatives on treatment with amines such as *N,N*-dimethylethylenediamine. Precursor ion scan spectra of the derivatives from a specific fragment ion due to C-N bond cleavage were measured. By use of this method, planar structure of tetrahydrofuranic acetogenins could be determined unambiguously. Absolute configuration of all carbinol centers of the isolated acetogenins were determined by advanced Mosher method and CD spectra. The concern about the amine method, feature of isolated compounds, biosynthesis, biological activity and other remaining problems were also discussed.

Contents

| | |
|-------------------------------------------------------------------------------------------------------------------------------------|-----|
| Chapter I . Introduction | 79 |
| 1. Natural product chemistry | 79 |
| 2. Annonaceous plants and <i>Annona squamosa</i> L. | 79 |
| 3. Phytochemical studies of annonaceous plants | 80 |
| 4. Annonaceous tetrahydrofuranic acetogenins | 81 |
| 5. Structure elucidation of tetrahydrofuranic acetogenins | 83 |
| 6. Purpose of this study | 86 |
| Chapter II . Application of a Precursor Ion Scanning Method for Planar Structure Elucidation of Tetrahydrofuranic Acetogenins | 87 |
| 1. Problems in structure elucidation of tetrahydrofuranic acetogenins and the precursor ion scanning method | 87 |
| 2. Derivatization of the lactone portion with amines and the structure determination of the derivatives | 91 |
| 3. Derivatization of squamocin (2) with benzylamine and its structure | 92 |
| 4. Precursor ion scanning of benzylamine derivative (4) | 93 |
| 5. Derivatization of squamocin (2) with <i>N,N</i> -dimethylethylenediamine | 94 |
| 6. Structure elucidation of <i>N,N</i> -dimethylethylenediamine derivatives (5) and (6) | 95 |
| 7. Precursor ion scanning of <i>N,N</i> -dimethylethylenediamine derivatives (5) | 97 |
| 8. Application of the amine method to other tetrahydrofuranic acetogenins | 98 |
| (1) Application of the amine method to squamocin-C (7) | 98 |
| (2) Application of the amine method to squamocin-F (8) | 99 |
| (3) Application of the amine method to squamostatin-C (9) | 100 |

*Department of Biological Safety

| | |
|--------------------------------------------------------------------------------------------------------|-----|
| (4) Application of the amine method to annonacin-10-one (10) | 101 |
| Chapter III. Structure Elucidation of New Tetrahydrofuranic Acetogenins | 104 |
| 1. The isolation procedure | 104 |
| 2. Structure elucidation of new tetrahydrofuran acetogenins | 106 |
| (1) Structures of squamocins-O ₁ (11), -O ₂ (12) | 106 |
| (2) Structure of squamosten-A (13) | 109 |
| (3) Structure of squamocin-N (14) | 112 |
| (4) Structure of squamocin-E (15) | 114 |
| (5) Structure of squamocin-B (16) | 115 |
| (6) Structure of squamostanal-A (17) | 116 |
| Chapter IV. Results and Discussion | 118 |
| 1. The amine method | 118 |
| 2. New tetrahydrofuranic acetogenins isolated from <i>Annona squamosa</i> L. seeds in this study | 119 |
| 3. Biosynthesis tetrahydrofuranic acetogenins | 120 |
| 4. Bioactivities of tetrahydrofuranic acetogenins | 122 |
| 5. Remained problems and a prospect in the future | 124 |
| Chapter V. Experimental Section | 125 |
| Acknowledgements | 139 |
| Appendices | 139 |
| Appendix A List of Abbreviations | 139 |
| Appendix B List of Figures | 140 |
| Appendix C List of Tables | 142 |
| References | 142 |
| Summary in Japanese | 148 |

Chapter I

Introduction

1. Natural product chemistry

We have been utilizing natural constituents produced by animals, plants and microorganisms as foods, perfumes, medicines, poisons, dyes, etc. since ancient age. Naturally occurring organic compounds from various organisms are often called "natural products". Generally speaking, natural products include proteins, hydrocarbons, lipids, and nucleic acids as principal components in a wide sense. However, targets in natural product chemistry are often compounds that are found in very small quantity and have various biological functions in living bodies, for example, hormones, pheromones, toxins, antibiotics, etc. These are classified as, carbohydrates, nucleic acid derivatives, amino acids and peptide, fatty acids and their derivatives, terpenoids, phenolics, polyketide, and alkaloids, on the basis of their structural and biosynthetic features (Mann et al., 1994; Torssel, 1983). A study of so-called "natural product chemistry", nowadays, comprises of isolation, structure elucidation, reactivity, synthesis, biosynthesis and bioactivity of natural compounds and their synthetic analog.

The history of natural product chemistry began at late eighteenth century, and has since been together with the development of organic chemistry. Therefore, natural product chemistry will be regarded as an origin of modern organic chemistry. Current natural product chemistry progressed drastically in 1960's in accordance with developments of various separation techniques (e.g. column chromatography (CC), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC), electrophoresis, ion exchange chromatography, etc.) and analytical methods (especially, nuclear magnetic resonance (NMR) spectrum, infrared (IR) spectrum, mass spectrum (MS), ultra violet (UV) spectrum, and X-ray crystallography, etc.). Furthermore, recent computer assisted analysis method have been grown to practical use levels for reactions, configuration analyses, etc.

Our knowledge of natural products are indispensable for improvement of the quality of our lives, and continuing efforts toward discovering novel compounds for important biological activities are necessary.

2. Annonaceous plants and *Annona squamosa* L.

Annonaceae is a large family of tropical and subtropical trees, shrubs and lianas comprising *ca.* 130 genera and more than 2300 species (Coner, 1969; Hotta et al., 1989). Some important features of the family are:

- (1) edible fruits; custard apple, sugar apple, atemoya, cherimoya, pawpaw, soursop etc.,
- (2) medicine; vermicide, abortifacient, insecticide, extermination of lice, etc.,
- (3) oils for food or material of soap,
- (4) material of Iran perfumery.

Some of annonaceous fruits, sugar apple and cherimoya, are imported in Japan for both fresh eating and processed consumption like sherbet and juice. Some of these are recently cultivated in Okinawa prefecture.

Many members of annonaceae are used in folk medicine for antiparasitic or antitumoral treatment of intestinal diseases. However, its phytochemical study has not been extensively carried out due to its distribution in tropical or subtropical area. In recent years, many interesting compounds have been reported (Leboeuf et al., 1982), and have gained organic chemist's and biochemist's attention because of their novel structure, and wide-range of bioactivities.

Characteristic features (Hayashi et al., 1985; Nishioka, 1998; Iwasa, 2001) of *Annona squamosa* L. (Japanese name: banreishi, shakatou) used in this study are

- (1) 5 ~ 6 m height,
- (2) short-stalked lance-shaped to oblong leaves 5 ~ 13 cm long and 2 ~ 5 cm broad, alternate in two rows,
- (3) yellow-green narrow flowers 1.5 ~ 2.5 cm long with three narrowly oblong petals, usually a few in a lateral cluster,
- (4) nearly round or heart-shaped yellow-green rugged fruits 7 ~ 8 cm in diameter, covered with a whitish bloom which soon turns blackish when rubbed or bruised, and is composed of numerous rounded tubercles or raised segments, with whitish, sweet, juicy and creamy pulp (Fig. 1 (Iwasa, 2001)).

The fruits are called as Buddha's head after its shape in India, and are commonly known as custard apple, sugar apple, sweet sop or atemoya in other part of the world. Original habitat of the *A. squamosa* L. is considered to be in West-Indian-islands, but it is cultivated as well in and around tropical area today (Coner, 1969; Hayashi et al., 1985; Nishioka, 1998; Iwasa, 2001).

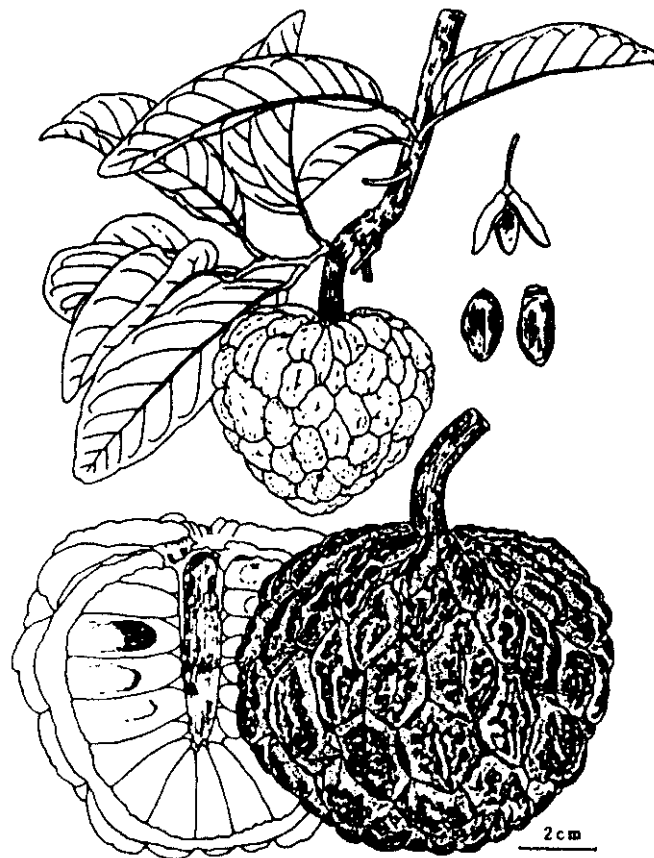


Fig. 1. *Annona squamosa* L.

3. Phytochemical studies of annonaceous plants

Annonaceous plants have been used as medicinal plants and the powder of seeds and leaves have been used for a prevention of lice in India (Hotta et al., 1989; Coner, 1969).

In the past, several compounds (terpenoids, alkaloids, etc.) exhibiting cytotoxicity, antitumor, insecticide, antibiotic, antifeedant, immunosuppressant, etc. have been isolated from annonaceous plants, and these were reviewed by Leboeuf et al. in 1982. Their review details the isolation of various alkaloids, carbohydrates, lipids, amino acids, proteins, polyphenols, essential oils, terpenes and aromatic compounds typically found in these plants (examples (Laboef et al., 1982; Morita et al., 1999); Fig. 2). However, a series of compounds termed tetrahydrofuranic acetogenins (or

annonaceous acetogenins), the very new type of compounds, were not mentioned in the review. These acetogenins have gained much attention recently, because of its wide range of bioactive spectra.

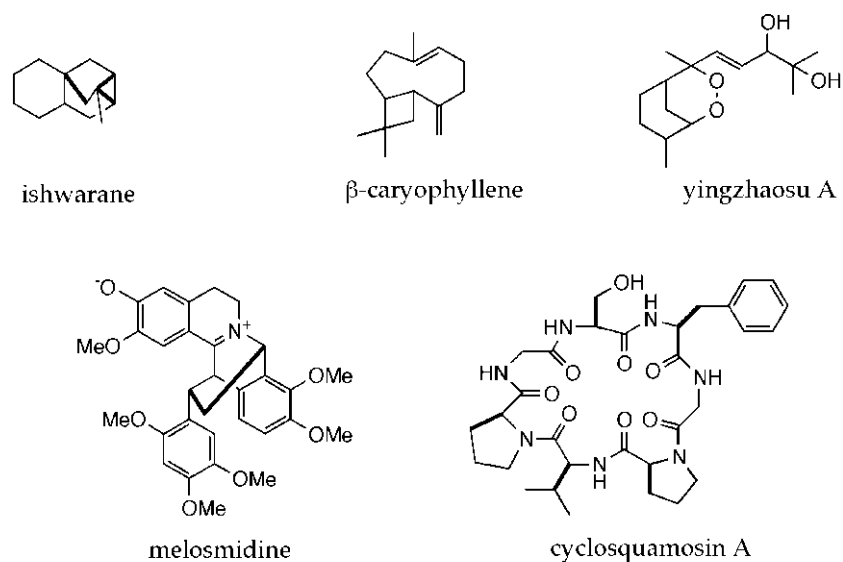


Fig. 2. Terpenoids and alkaloids found in annonaceae

4. Annonaceous tetrahydrofuranic acetogenins

The first tetrahydrofuranic acetogenin, uvaricin (**1**) isolated from roots of *Uvaria Acuminata*, was reported by Jolad et al. in 1982. This compound was a novel type of natural product, having methylene chain, an adjacent bis-tetrahydrofuran rings at center of the long methylene chain and an α,β -unsaturated- γ -lactone at its the terminal as a basic skeleton (Fig. 3). Since the discovery of uvaricin, over 350 tetrahydrofuranic acetogenins have been reported from limited genera of annonaceae (Table 1) (Rupprecht et al., 1990; Fang et al., 1993b; Gu et al., 1995; Zeng et al., 1996; Alali et al., 1999).

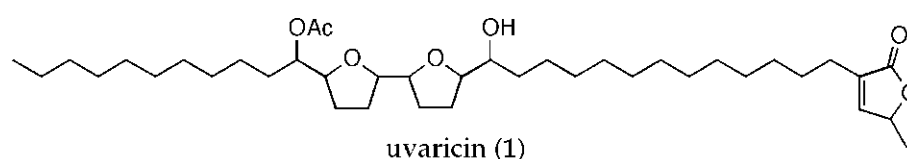


Fig. 3. Structure of uvaricin (**1**)

Table 1. Genera of source of tetrahydrofuranic acetogenins

| | |
|----------------------|---------------------|
| <i>Annona</i> | <i>Miliusa</i> |
| <i>Anomianthus</i> | <i>Opyrypetalum</i> |
| <i>Asimina</i> | <i>Rollinia</i> |
| <i>Disepalum</i> | <i>Uvaria</i> |
| <i>Goniothalamus</i> | <i>Xylopia</i> |

The fundamental structural features of tetrahydrofuranic acetogenins are:

- (1) Having hydrocarbon chain, of C35 or C37 in length.
- (2) 1 ~ 3 tetrahydrofuran rings are present.
- (3) One γ -lactone is present at an end of hydrocarbon chain.

(4) 2 ~ 8 hydroxyl groups (rarely carbonyl or acetoxy group) are present.

These acetogenins can be classified to five types according to the position of tetrahydrofuran ring (Fig. 4) ;

- (A) adjacent bis-tetrahydrofuran type
- (B) non-adjacent bis-tetrahydrofuran type
- (C) mono tetrahydrofuran type
- (D) tri-tetrahydrofuran type
- (E) non tetrahydrofuranic type.

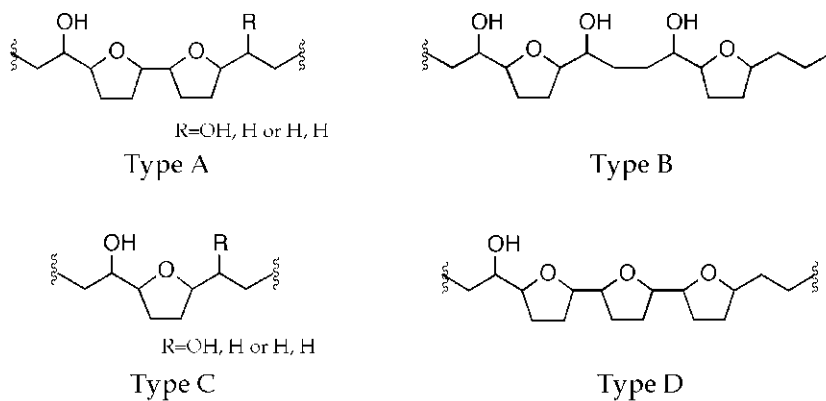


Fig. 4. Classification of tetrahydrofuran portion

Lactone portions of reported acetogenins are classified into four types as bellow) (Rupprecht et al., 1990; Fang et al., 1993b; Gu et al., 1995; Zeng et al., 1996; Alali et al., 1999).

- (a) α,β -unsaturated- γ -lactone
- (b) ketolactone type
- (c) γ -hydroxy lactone
- (d) β -hydroxy lactone

Lactones of type (a) are the most abundant. Lactones of type (b) are derived from γ -hydroxylated lactone of type

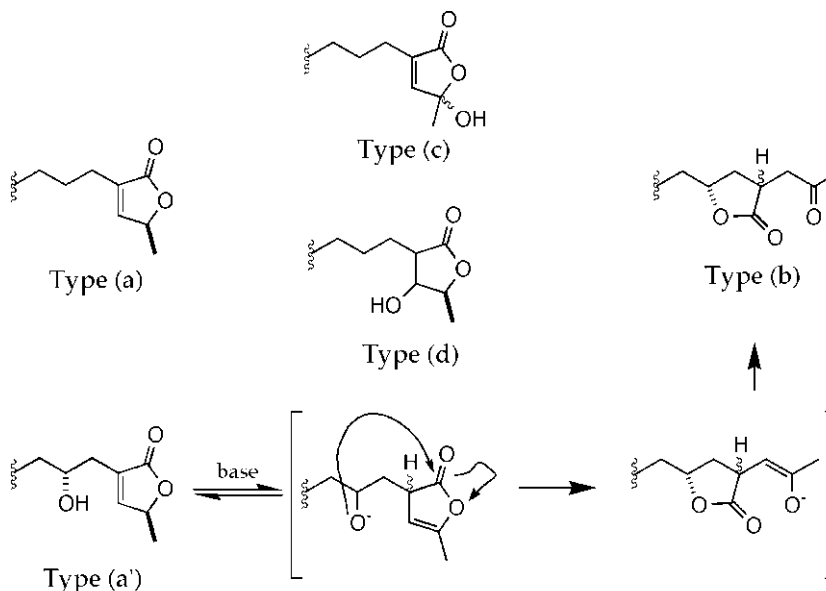


Fig. 5. Structures of lactone moiety

(a') via non-enzymatic process. McLaughlin's group reported that type (a') acetogenin could be transformed into the type (b) lactone when treated with a base (Fig. 5). Type (c) lactones are rare and type (d) lactone is considered as a Michael adduct of type (a) lactone.

As a structural example of squamocin (**2**) (Fujimoto et al., 1988) and squamostatin-A (**3**) (Fujimoto et al., 1990) isolated from *A. squamosa* L. seeds, by our research group are shown in Fig. 6. Hereafter, a methylene chain between α,β -unsaturated- γ -lactone and tetrahydrofuran will be called a-chain, the other will be called b-chain. Furthermore, a tetrahydrofuran close to γ -lactone will be called ring-A, the other in case of bis-tetrahydrofuran acetogenins, will be called ring-B (Fig. 6).

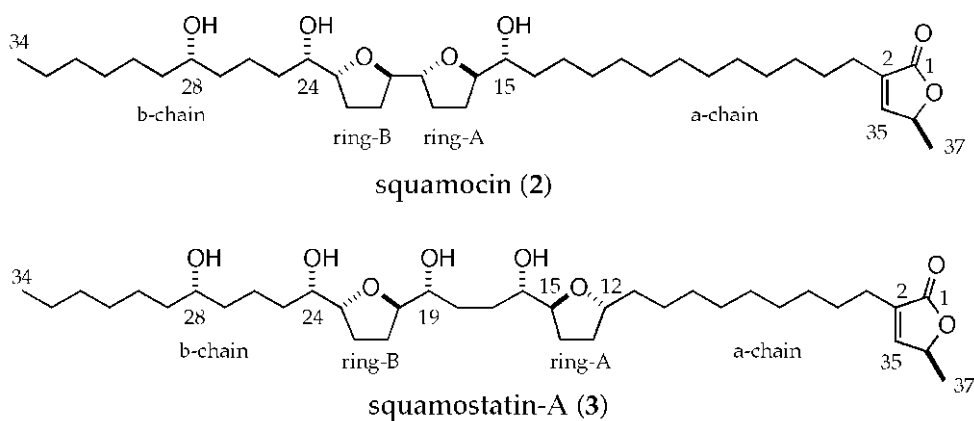


Fig. 6. Structures of squamocin (**2**) and squamostatin-A (**3**)

5. Structure elucidation of tetrahydrofuranic acetogenins

The structure of tetrahydrofuranic acetogenins seems to be rather simple, however, several incorrect structures or assignments have been reported. The reason for this is due to the following.

- (1) many stereo isomers (many asymmetric carbon) are present.
- (2) position of functional groups (tetrahydrofurans, hydroxyls, etc.) cannot be deduced easily with NMR. (because proton signals on oxymethylene carbon are significantly overlapped).
- (3) these acetogenins are not suitable for X ray crystallographic analysis because of its oily, amorphous or micro-crystal state (except for two derivative (Pettit et al., 1987; Born et al., 1990)).

The problems associated with structure elucidation using NMR of a series of the tetrahydrofuranic acetogenins are

- (1) determination of the position of tetrahydrofuran rings and hydroxyl groups (planar structure),
- (2) determination of the stereochemistry (relative and absolute stereochemistry).

It is therefore essential to analyze not only NMR, but also other analytical method as well, such as MS spectra. The points of structure elucidation will be described bellow according to their types: (A), (B), (C), (D) and (E).

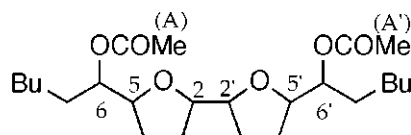
Type (A) (adjacent bis-tetrahydrofurans): This type of acetogenin is the second largest group among the tetrahydrofuranic acetogenins. A few acetogenins lacking one of hydroxyl group adjacent to a ring-A were reported (Gu et al., 1997; Shi et al., 1997a). Most of acetogenins isolated from *A. squamosa* L. in this study were classified to this class.

There are six asymmetric carbons around the bis-tetrahydrofuran moiety. Hoye et al. established a method (Hoye's rule) to determine its relative stereochemistry by comparison of $^1\text{H-NMR}$ data between per-acetylated aceto-

genin and acetylated model compound in 1987 (Table 2) (Hoye and Suhadolnik, 1987; Hoye and Zhuang, 1988). Recently, our research group (Sahai et al., 1994) and McLaughlin's (Rieser et al., 1992) established their absolute stereochemistry with the advanced Mosher method by using their α -methoxy- α -(trifluoromethyl)phenylacetyl (MTPA) esters (as described in Chapter).

Table 2. Hoye's rule: chemical shifts of diagnostic protons of model bis-tetrahydrofuran diacetates

| | | | | | | | | | |
|---------------------|-----|-----|-----|-------|-----|-----|-----|------------|-----------|
| <i>er/c/th/c/er</i> | 6 | | | | 5 | 2 | | | A |
| <i>er/t/th/c/er</i> | 6' | | | | 5 | 5' | 2 | 2' | AA |
| <i>er/t/th/t/er</i> | 6 | | | | 5 | 2 | | | A |
| <i>er/c/er/c/er</i> | 6 | | | | 5 | | 2 | | A |
| <i>er/t/er/c/er</i> | 6 | 6' | | | 5 | 5' | 2' | | AA |
| <i>er/t/er/t/er</i> | 6 | | | | 5 | | 2 | | A |
| <i>th/c/th/c/th</i> | 6 | | | | 5 | 2 | | | A |
| <i>th/t/th/c/th</i> | 6' | | | 5 | 25 | 2' | | | AA |
| <i>th/t/th/t/th</i> | 6 | | | | 5 | | 2 | | A |
| <i>th/c/er/c/th</i> | 6 | | | | 5 | | 2 | | A |
| <i>th/t/er/c/th</i> | 6' | | | | 5 | 5' | 2' | | AA |
| <i>th/t/er/t/th</i> | 6 | | | | 5 | | 2 | | A |
| δ_H | 5.0 | 4.9 | 4.8 | ~ 4.1 | 4.0 | 3.9 | 3.8 | 3.7 ~ 2.08 | 2.06 2.04 |



* The left column indicates 6:5/5:2/2:2'/2':5'/5':6' stereorelationships.

Type (B) (non-adjacent bis-tetrahydrofurans): This type of acetogenin contains two non-adjacent tetrahydrofuran rings, which are always separated by a four-carbon chain. One of the tetrahydrofuran ring (ring-A) is flanked by one hydroxyl group which is positioned between the two tetrahydrofuran rings, while the other tetrahydrofuran ring (ring-B) is flanked by two hydroxyl groups (Fig. 4). Due to the presence of seven asymmetric carbon centers around tetrahydrofurans, it is significantly difficult to determine the structure of these acetogenins, and several incorrect structures have been reported (Laprevote et al., 1991; Lios et al., 1989; Nonfon et al., 1990). The relative stereochemistry between tetrahydrofurans and hydroxyls were determined by means of Hoye's rule (Table 2) and/or Born's rule (Table 3). Lately, our group solved the absolute stereochemistry of this type acetogenins by means of the advanced Mosher method using synthetic model compounds (Shimada et al., 1994).

Table 3. Born's rule: chemical shifts of diagnostic protons and carbons of model tetrahydrofurans

| | H-1/C-1 | H-2/C-2 |
|--|------------|------------|
| | 3.84/71.83 | 3.84/82.29 |
| | 3.40/73.87 | 3.79/82.47 |

Type (C) (mono-tetrahydrofuran): This type of acetogenins is the largest group of tetrahydrofuranic acetogenins. They have only one tetrahydrofuran ring and two (C1) or one (C2) hydroxyls flanked to the tetrahydrofuran (Fig. 4). These structures can be distinguished easily from each other by ^{13}C -NMR data (Fig. 7).

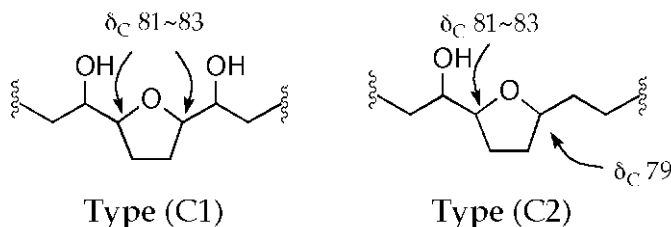


Fig. 7. Difference between C1 and C2 in ^{13}C -NMR

The relative stereochemistry can be determined easily by comparison of NMR data with model compounds (Gale et al., 1993a, 1993b). The determination of absolute stereochemistry with advanced Mosher method was reported by McLaughlin's group recently (Rieser et al., 1994).

Type (D) (tri-tetrahydrofurans): This type of acetogenin has an adjacent tri-tetrahydrofuran rings core, only reported for goniocin (Gu et al., 1994a) (Fig. 8). The relative stereochemistry was assigned to *trans/threo/trans/threo/trans/threo* by comparing ^1H - and ^{13}C -NMR data of other acetogenins. The Absolute chemistry was determined as *4R,10S,13R,14R,17R,18R,21R,22R,37S* by advanced Mosher method.

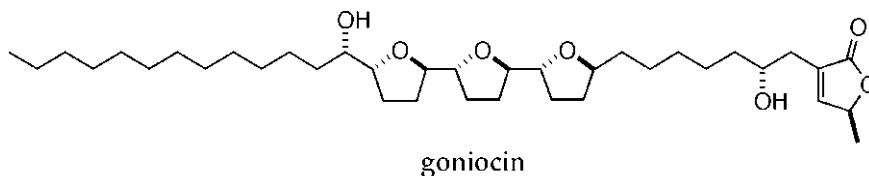


Fig. 8. Structure of goniocin from *Goniotalamus giganteus* bark

Another class of acetogenins is called type (E) (non-tetrahydrofuran). Some of examples (Fang et al., 1993a; Saizarbitoria et al., 1995; Gleye et al., 1999) are shown in Fig. 9. This subclass probably represent the biogenetic precursors of more complex acetogenins, as previously proposed by Rupprecht et al. (Rupprecht et al., 1990; Fang et al., 1993b; Gu et al., 1995; Zeng et al., 1996; Alali et al., 1999).

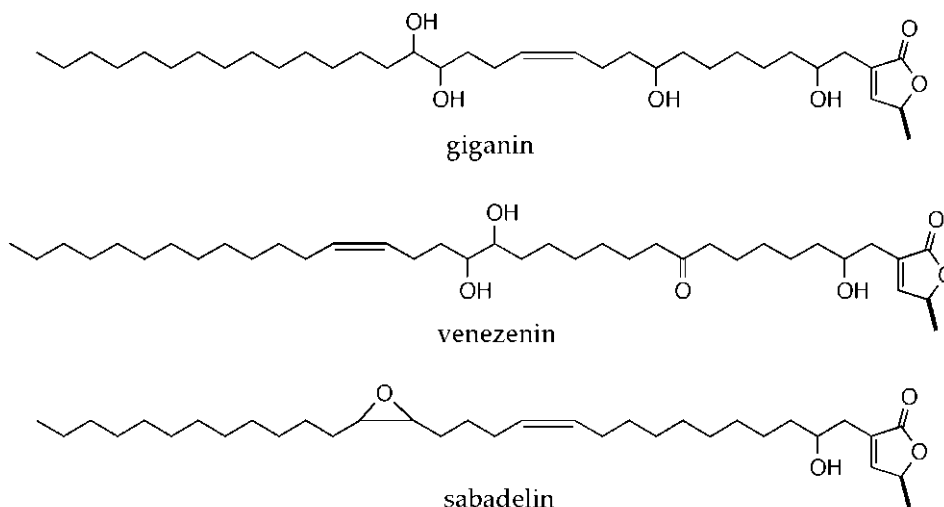


Fig. 9. Examples of acetogenins of type (E)

In recent years, unusual acetogenins were reported. These contain novel functional groups, a tetrahydropyran ring (Shi et al., 1995, 1996), a tetrahydrofuran not flanked to a hydroxyl group (Alfonso et al., 1996; Chen et al., 1999) and a fatty acid ester (Gleye et al., 1998).

6. Purpose of this study

In general, the MS analysis is essential to determine a planar structure since other spectroscopic method (NMR, IR and UV) give insufficient information as mentioned above. Information provided by fragmentation pattern can only identify the position of tetrahydrofurans and hydroxyl groups. Fragmentation of fission around tetrahydrofurans are observed clearly in EI-, CI- and FAB-MS spectra, but fission of alpha or beta to hydroxyls on hydrocarbon chain are not observed clearly (refer to Chapter). The MS spectra of some derivatives (*e.g.* acetate, TMS ether) have not been so effective, either.

In this study, these problems were solved with combination of derivatization with *N,N*-dimethylethylenediamine and the precursor ion scanning method (FAB-MS/MS). Planar structure of acetogenins can be determined with this new method easily (refer to Chapter). In Chapter , the structure determination of seven new acetogenins from *A. squamosa* L. seeds will be described. The results of the new method for determination of planar structure, biosynthesis, bioactivities, and a prospect in the future about tetrahydrofuranic acetogenins will be discussed in Chapter .

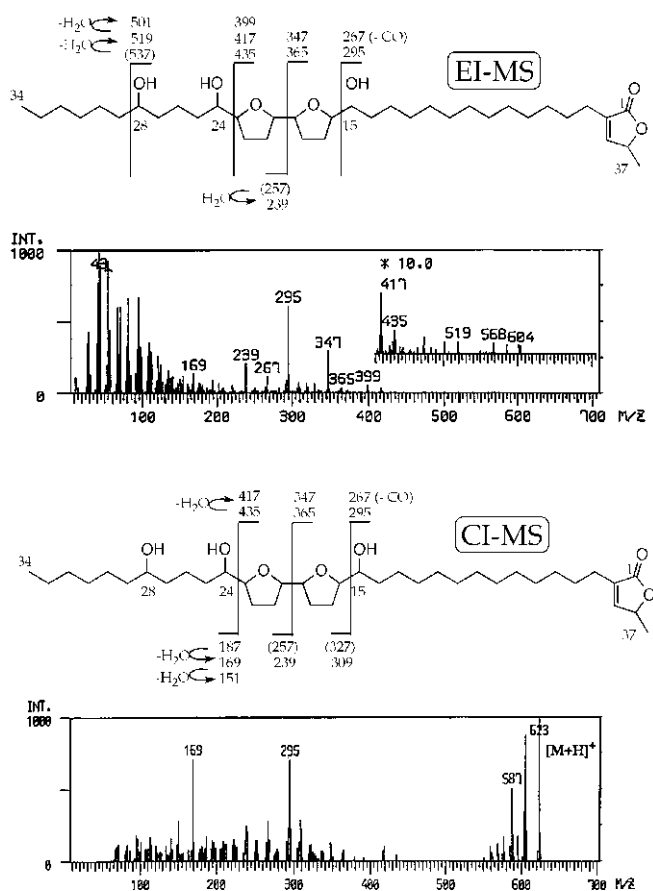
Chapter

Application of a Precursor Ion Scanning Method for Planar Structure Elucidation of Tetrahydrofuranic Acetogenins

1. Problems in structure elucidation of tetrahydrofuranic acetogenins and the precursor ion scanning method

The major difficulty associated with the structure elucidation of a new tetrahydrofuranic acetogenin lies in the location of the different oxygenated functional groups (tetrahydrofuran, hydroxyl, acetoxy, ketone etc.) on the hydrocarbon chain. As mentioned in Chapter I, MS spectral analysis is essential to determine the position of functional groups, which generally cannot be determined by other spectral method (except for oxygenated groups close to terminus).

The electron ionization mass spectrometry (EI-MS), chemical ionization mass spectrometry (CI-MS) and fast atom bombardment mass spectrometry (FAB-MS) of squamocin (**2**) were shown in Fig. 10 as an example. The fragment ion peaks in parentheses in the figure were not observed clearly. The fragment ions resulting from fission of tetrahydrofurans were observed at high intensity, and position of tetrahydrofurans can be easily determined. On the other hand, fragment ions indicating the position of hydroxyl groups on the hydrocarbon chain were generally observed at weak intensity and these were difficult to distinguish from background signals. Some research groups reported several incorrect structures by various MS analysis, which structures were apparently not supported by their NMR spectral data (Laprevote et al., 1991; Santos et al., 1996). It is, thus, noteworthy that particular care must be taken to analyze MS spectra, because accompanying dehydration fragmentation makes interpretation difficult.



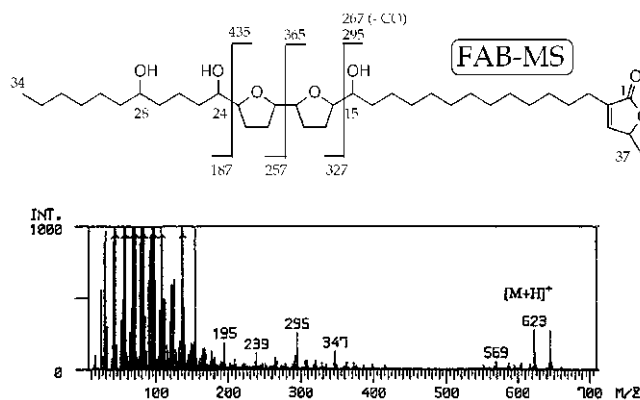


Fig. 10. EI-, CI- and FAB-MS spectra of squamocin (2)

Derivatization to acetate, trimethylsilyl (TMS) ether can not solve this problem. The FAB-MS spectrum of squamocin acetate is shown in Fig. 11. This chart indicates that derivatization was not enough to gain useful signal/noise ratio. Some researchers often used stable isotope derivatives; d_3 -acetate, d_9 -TMS ether, for confirmation of position of the hydroxyl groups, where fragment ions of α -fission of hydroxyls were detected even for small intensity (McCloud et al., 1987; Alkofahi et al., 1988). But, in general, this problem could not be solved easily, since these ion intensities were too weak.

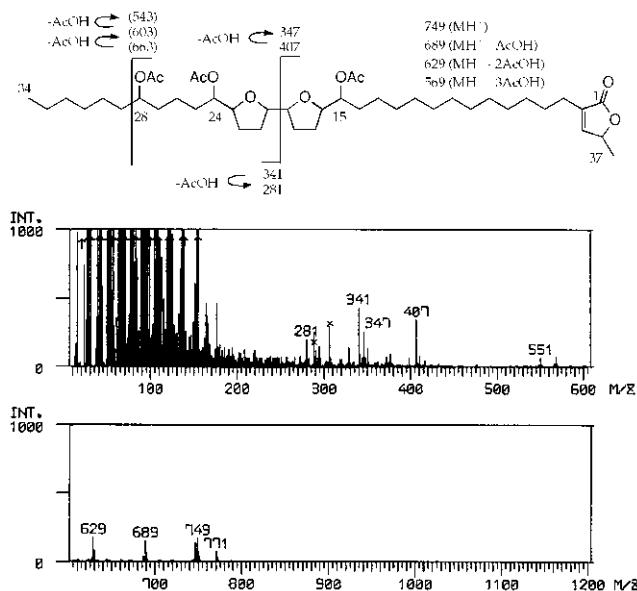


Fig. 11. FAB-MS spectrum of squamocin acetate

Alternatively, tandem mass spectrometry (FAB-MS/MS) has been tried to solve the problem. The MS/MS has been mainly applied for identification of amino acid sequences of peptides, sugars and nucleic acids. The product ion scanning method has been used principally for the purpose of structure determination (Desipderio, 1991).

In the product ion scanning method (also known as daughter ion scanning method), a specific ion (parent ion, precursor ion) derived from first stage of MS is refragmented with helium gas etc., and then fragment ions (daughter ion, product ion) produced in second stage of MS are measured (left side in Fig. 12). When this method were applied to tetrahydrofuranic acetogenins, fragment ions containing the lactone unit were observed at strong intensities.

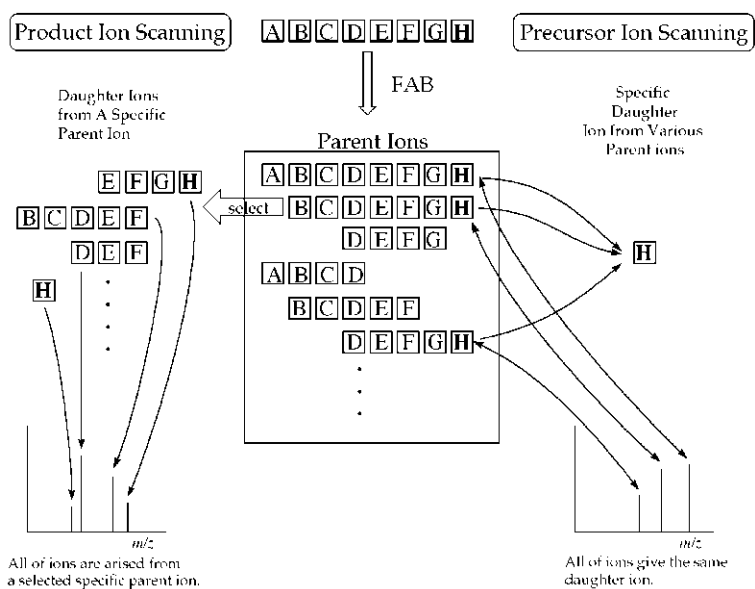
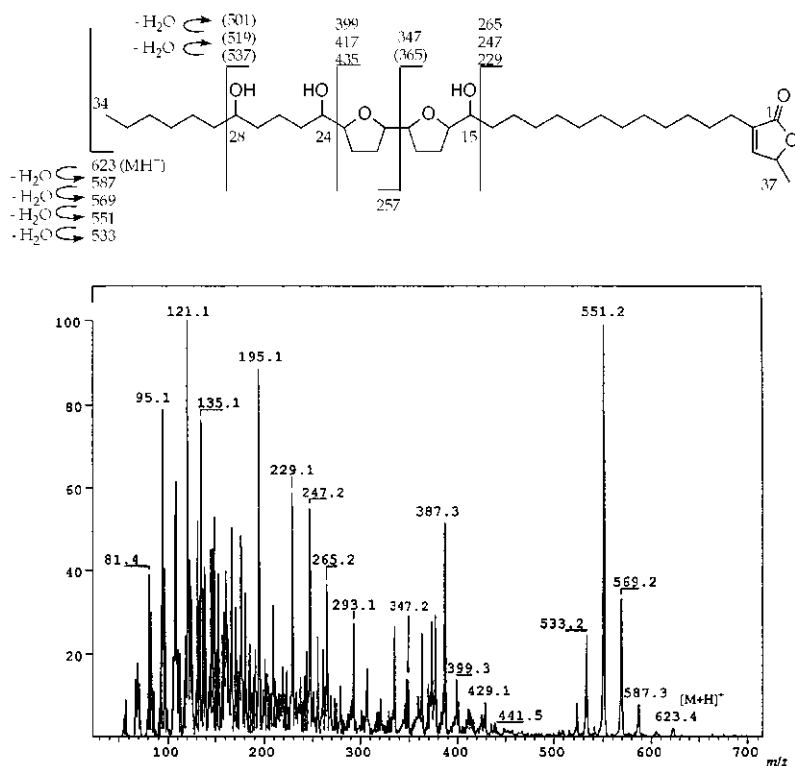


Fig. 12. The image of product and precursor ion scanning method

At first, product ion scanning of m/z 623 (MH^+) was performed to squamocin (**2**) as a model acetogenin (Fig. 13). The diagnostic ions indicating existence of hydroxyl group at C-28 (m/z 537 (C-28/C-29), 519 (C-28/C-29 - H_2O), 501 (C-28/C-29 - $2H_2O$)) were not observed clearly. It is probably due to the facile dehydration in the second fragmentation, as 1-5 dehydration peaks from the molecular ion (m/z 623 (MH^+), 605 ($MH^+ - H_2O$), 587 ($MH^+ - 2H_2O$), 569 ($MH^+ - 3H_2O$), 551 ($MH^+ - 4H_2O$), 533 ($MH^+ - 5H_2O$)) were seen.

Fig. 13. Product ion scanning from m/z 623 for squamocin (**2**)

Ruprevote et al. reported an application of CI-MS/MS to a (B) type acetogenin, but the planar structure that they reported was inconsistent with the NMR data (Laprevote et al., 1991). These results indicate that adaptation of product ion scanning requires many scanning from different parent ions and requires systematic analysis. Next, the precursor ion scanning method was tested.

The precursor ion scanning method (also called as parent ion scanning method) can detect precursor ions (parent ions) in the first stage of MS which produce a specific product ion (daughter ion) in the second stage of MS (right side in Fig. 12). If a specific ion derived from a specific partial structure is produced, fragment ions containing the specific structure unit can be effectively detected by using this method. The application of this method will make the analysis very easy, and simultaneously gain better S/N ratio.

The precursor ion scanning from m/z 97, the fragment ion derived from an α,β -unsaturated- γ -lactone moiety, which was detected as an intense peak in FAB-MS spectra (Fig. 10), was conducted for squamocin (**2**). The MS chart of this experiment is shown in Fig. 14. However, the diagnostic ions such as m/z 537 (C-28/C-29 - H_2O), 519 (C-28/C-29 - H_2O), 501 (C-28/C-29 - $2H_2O$) which indicate an existence of 28-OH on b-chain, were not detected clearly.

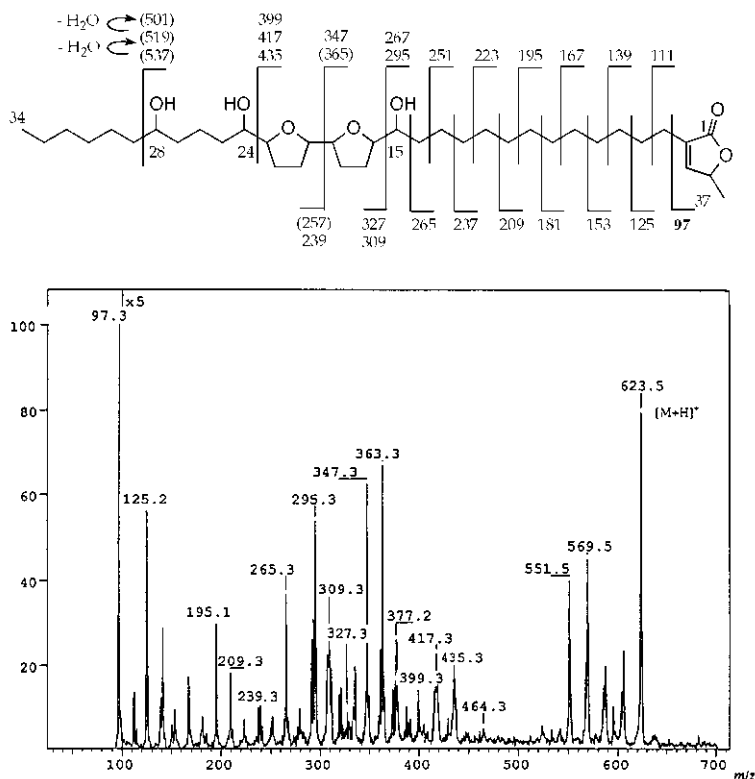
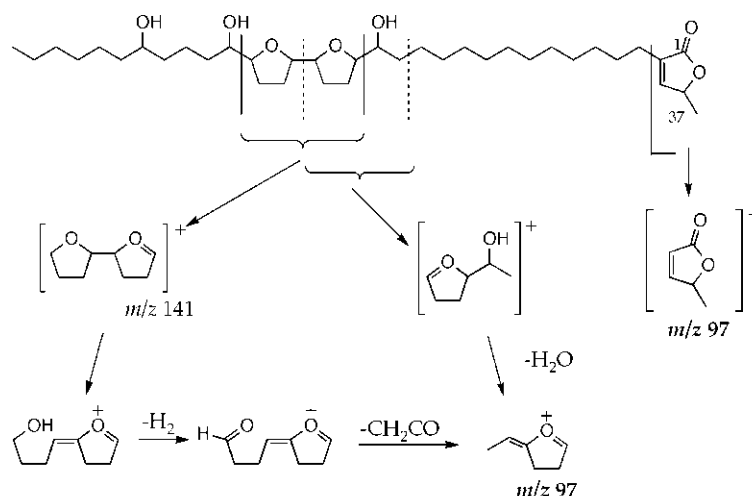


Fig. 14. Precursor ion scanning from m/z 97 for squamocin (**2**)

On the other hand, ions m/z 327 (C-15/C-16), 309 (C-15/C-16 - H_2O), 239 (C-19/C-20 - H_2O) derived from the adjacent tetrahydrofurans were observed in this spectrogram. It was found that the m/z 97 ion can be also derived from the tetrahydrofurans portion too (Fig. 15) (Jolad et al., 1982). This result shows that the ion derived from tetrahydrofuran reduce S/N ratio of the ion derived from the lactone moiety. Therefore, derivatization of the lactone portion was required.

Fig. 15. Formation of m/z 97 fragment ion

2. Derivatization of the lactone portion with amines and the structure determination of the derivatives.

To improve the former method, derivatization of terminal lactone was attempted. The derivative should produce a specific m/z fragment ion, not observed in other portion.

An example of such derivatization widely used for the precursor ion scanning (Vath et al., 1988) is shown in Fig. 16. Carboxyl terminal of the peptide is often derivatized and transformed to a new functional group with a quaternary ammonium group. The resulting new functional group produces characteristic fragment ions m/z 44 and 58.

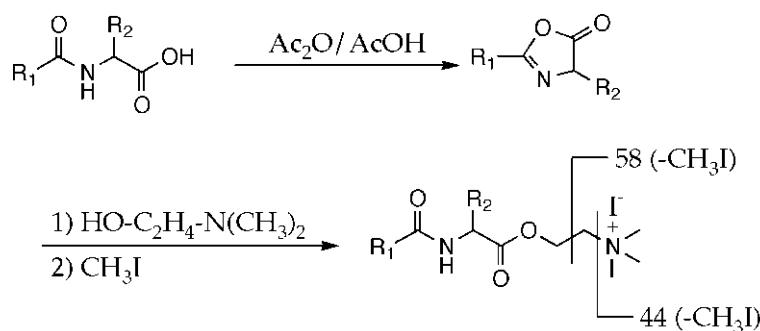
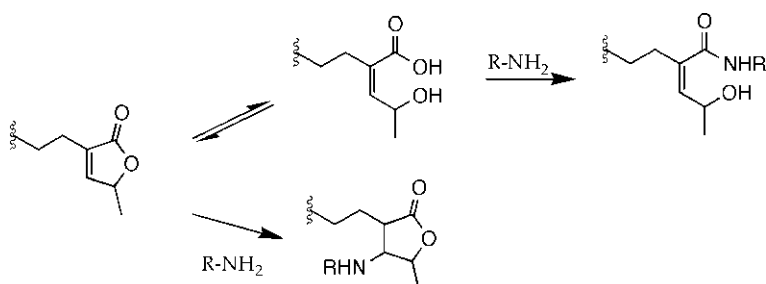


Fig. 16. Example of the derivatization for mass analysis of peptides

An α,β -unsaturated- γ -lactone moiety can be assumed as a γ -hydroxy acid, and this functional group reacts with amines easily (Fig. 17). Furthermore, α,β -unsaturated- γ -lactone can act as Michael acceptor (Fig. 17). It is noteworthy that protection of other functional group of the acetogenin is not necessary in this reaction. At first, derivatization with benzyl amine (MW: 107) was attempted. A newly derived functional group produced a specific strong fragment ion at m/z 91. It is well known that α -fission at benzyl position occurred easily because of formation of a stable benzyl cation.

Fig. 17. Predictable reaction of α,β -unsaturated- γ -lactone with amines

3. Derivatization of squamocin (**2**) with benzylamine and its structure

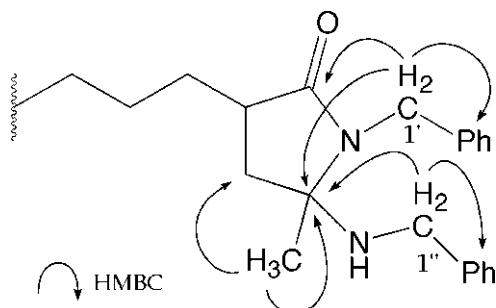
Benzylamine 50 μg (excess), squamocin (**2**) 50 mg was sealed in a micro tube, and heated. This reaction was proceeded at 90 °C for twenty hours. The amine was removed by a pumping with a vacuum pump under heating (at 70 °C), and purification (silica gel chromatography ($\text{CHCl}_3/\text{AcOEt}/\text{MeOH} = 2 : 3 : 1$) and ODS HPLC (MeOH)) afforded reactant (**4**) more polar than the parent material on the silica gel TLC (see experimental section).

The molecular weight and molecular formula for the derivative (**4**) were obtained as 818, and $\text{C}_{51}\text{H}_{82}\text{O}_6\text{N}_2$ from FAB-MS and HR-FAB-MS respectively. It was considered that **4** was formed by reaction with two molecules of benzylamine. In IR spectra, an absorbance of 1760 cm^{-1} (α,β -unsaturated- γ -lactone) disappeared, and a new absorbance was observed at 1670 cm^{-1} indicating γ -lactam. The ^1H - and ^{13}C -NMR experiments (Table 4) made it clear that **4** was an diastereomeric mixture of **4a** and **4b**, and proved the disappearance of a double bond and the presence of two benzyl

Table 4. NMR data of benzylamine derivative (**4**)

| | 4a (major) | | 4b (minor) | |
|-----|-------------------------------|---------------------|-------------------------------|---------------------|
| | δ_{H} | δ_{C} | δ_{H} | δ_{C} |
| 1 | - | 176.39 | - | 177.2 |
| 2 | 2.55 (m) | 41.00 | 2.55 (m) | 42.05 |
| 3 | buried | 32.51 | buried | 32.51 |
| 4 | buried | 27.40 | buried | 27.40 |
| 35 | ~1.3 (m) | 36.77 | ~1.3 (m) | 36.77 |
| | ~2.0 (m) | | ~2.0 (m) | |
| 36 | - | 77.11 | - | 77.38 |
| 37 | 1.22 (s) | 27.49 | 1.22 (s) | 27.49 |
| 1' | 4.25 (d, $J=15.8\text{ Hz}$) | 45.65 | 4.34 (d, $J=14.9\text{ Hz}$) | 46.43 |
| | 4.62 (d, $J=15.3\text{ Hz}$) | | 4.57 (d, $J=16.2\text{ Hz}$) | |
| 1'' | 3.26 (d, $J=12.7\text{ Hz}$) | 42.28 | 3.24 (d, $J=12.7\text{ Hz}$) | 42.05 |
| | 3.62 (d, $J=13.2\text{ Hz}$) | | 3.51 (d, $J=13.2\text{ Hz}$) | |

*This Table lists the data around lactam.

Fig. 18. Structure of benzylamine derivative (**4**)

groups. ^{13}C -NMR spectra analysis further showed a new *N,N*-acetal (aminal) carbon signal *ca.* δ_c 76. An additional analysis of H-H-COSY, C-H-COSY and HMBC confirmed **4a** and **4b** structures shown in Fig. 18. The ratio of **4a** to **4b** was *ca.* 3 : 1 from the intensity of the spot on TLC and ^{13}C -NMR signal intensity.

4. Precursor ion scanning of benzylamine derivative (4)

The FAB-MS spectrum of derivative (**4**) is shown in Fig. 19. Since the intense fragment ion of m/z 91 was observed as expected, precursor ion scanning from m/z 91 was performed.

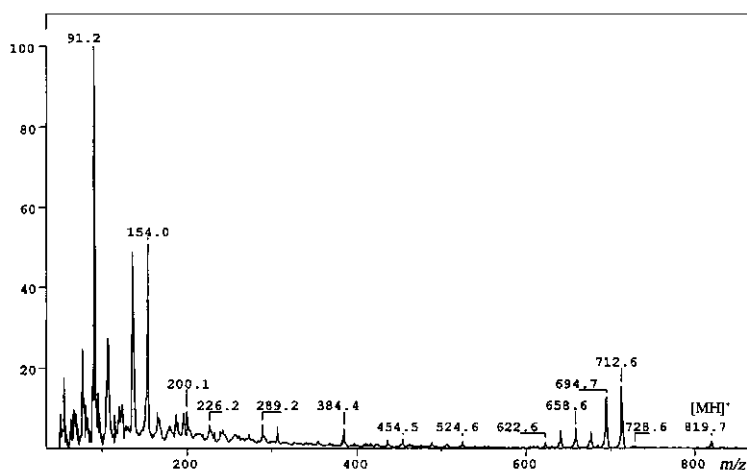


Fig. 19. FAB-MS spectrum of the benzylamine derivative

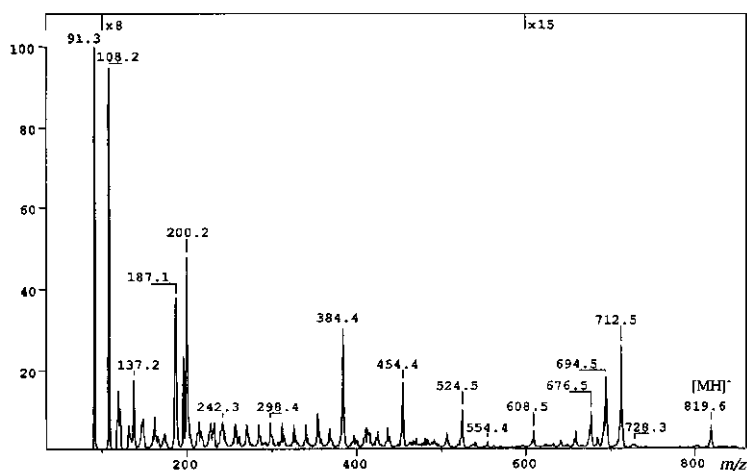
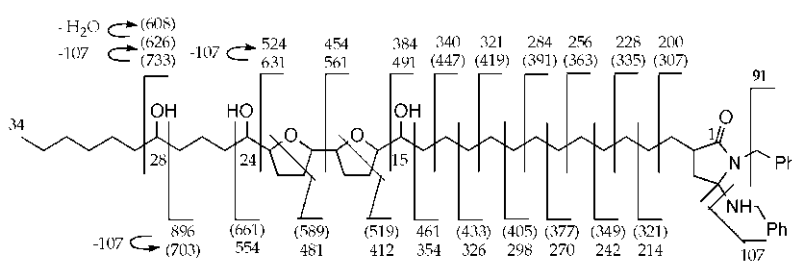


Fig. 20. Precursor ion scanning from m/z 91 for the benzylamine (**4**)

The detection of undesirable m/z 91 ion, not derived from lactam portion, was suppressed at low level (Fig. 20), and S/N ratio of fragment ions containing a terminal amine increased. In these results, almost all the fission of carbon-carbon bonds was observed, and the planar structure of the squamocin (**2**) was elucidated unequivocally. This result indicated that this derivative can be applicable to this type of compounds. Furthermore, another possibility for derivatization was investigated, as it appeared that a small amount of m/z 91 ion was still contaminating from other portion.

5. Derivatization of squamocin (**2**) with *N,N*-dimethylethylenediamine

Tetrahydrofuranic acetogenins generate odd number of fragment ions in FAB-MS generally, because these compounds are composed of carbon, hydrogen and oxygen atoms. Thus, it seemed that precursor ion scanning from even number fragment ions will be quite useful.

Squamocin (**2**) was then reacted with *N,N*-dimethylethylenediamine (Fig. 21). This reaction was performed in a neat condition: **2** (10 mg), *N,N*-dimethylethylenediamine (5 μ l, excess) were sealed in a microtube, and then heated at 80 °C for four hours. Two new compounds (**5a**) and (**5b**) (2 : 1) more polar than **2** were observed on a silica gel TLC. These were purified on silica gel pTLC [20% yield (**5a** + **5b**). Major compound (**6**) could not be observed at this time, because of overlap around the origin with the strong reagent band.]. Next, this mixture was developed on a silica gel TLC two dimensionally, and non-diagonal spots were observed (Fig. 22), indicating that **5a** and **5b** were isomerized to each other and could not be separated.

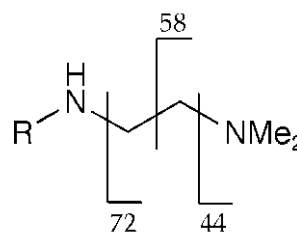
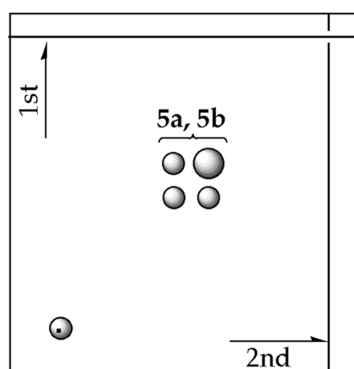


Fig. 21. Expected fragment ions of *N,N*-dimethylethylenediamine derivative in FAB-MS



*Developing solvent:
EtOAc/CHCl₃/MeOH = 2 : 3 : 1
**Second development was performed
after first development simultaneously.

Fig. 22. Two dimensional TLC experiment of derivative (**5**)

In order to obtain high yield for further analysis, squamocin (**2**) was treated in large amount under the same condition, followed by separation on silica gel column chromatography, which afforded **5a** and **5b** (AcOEt/MeOH = 1 : 1) (yield: 20%). However, **5a** and **5b** were also detected in a following fraction eluted with elution solvent (AcOEt/MeOH = 1 : 2) (total yield: 50%). This result suggested a possibility of decomposition of more polar major component into **5a** and **5b**. For the purpose of solving these compounds behavior, an excess reagent was removed with a vacuum pump under warming (ca. 60 °C). The resulting crude mixture was developed two dimensionally on a silica gel TLC. When the second development was performed immediately after first development, decomposition was not observed (It was seen as same as Fig. 22). However, when the first development was left for 8-hours, the unidentified compound (**6**) changed to **5a** and **5b** (Fig. 23). The compound **6** was detected as a single spot on the TLC after

developing with MeOH/2N-aqueous ammonia 7 : 3 (Rf value: 0.35). Purification of **6** with an octadecyl silica gel (ODS) column chromatography was also tried, but **6** could not be eluted with methanol. Consequently, structural analysis of **6** was carried out from a semi-pure sample, after removing the amine with vacuum pump.

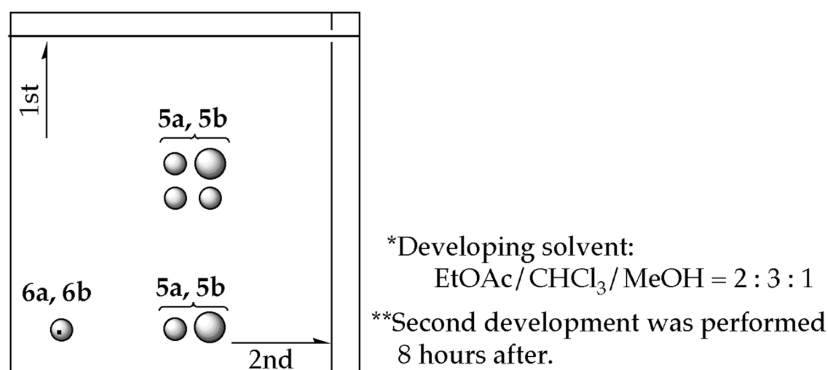


Fig. 23. Two dimensional TLC experiment of crude reaction mixture

The derivatization was also tried in dioxane. Squamocin (**2**) 10 mg, *N,N*-dimethylethylenediamine 0.5 μ l, dioxane 0.5 ml were sealed in a microtube, and heated at 80 $^{\circ}$ C for eight hours. The TLC experiment proved that the reactant contained **2** (ca. 50 %) and **5a, 5b** (ca. 50 %), but not **6**.

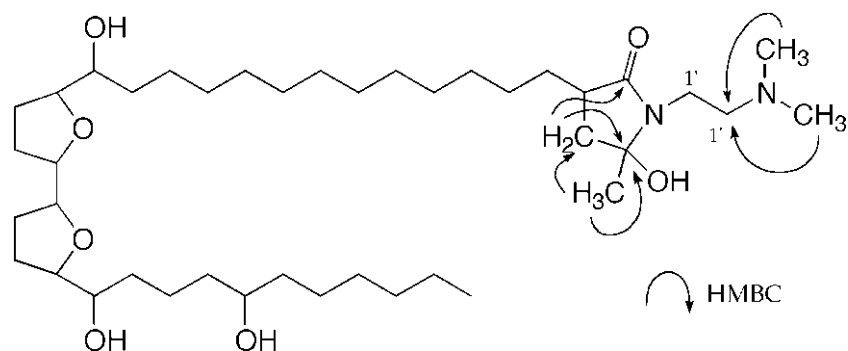
6. Structure elucidation of *N,N*-dimethylethylenediamine derivatives (**5**) and (**6**)

The molecular ion peak (MH⁺) *m/z* 711 in HR-FAB-MS indicated that the derivative (**5a, 5b**) had a molecular formula C₄₁H₇₈O₇N₂. From this composition, it was estimated that **5a** and **5b** were formed by reaction with one mole of *N,N*-dimethylethylenediamine. In IR spectra, an absorbance at 1760 cm⁻¹ for α,β -unsaturated- γ -lactone disappeared, and a new absorbance at 1670 cm⁻¹ for γ -lactam was observed. The ¹H- and ¹³C-NMR (containing INEPT experiment) (Table 5) revealed that the double bond in γ -lactone moiety disappeared, and new methylene and methyne carbons (2C) were formed. Further, analyses by H-H-COSY, C-H-COSY and HMBC spectra identified the structure of **5a** and **5b** having an amina structure as depicted in Fig. 24. The ratio of **5a** to **5b** was ca. 2 : 1 from the signal intensity of ¹³C-NMR and TLC.

Table 5. NMR data of the amine derivative (**5**)

| | 5a (major) | | 5b (minor) | |
|------|-------------------------------------------------------------------------|---------------------|-------------------------------------------------------------------------|---------------------|
| | δ_{H} | δ_{C} | δ_{H} | δ_{C} |
| 1 | - | 177.64 | - | 176.74 |
| 2 | 2.68 (m) | 39.94 | 2.68 (m) | 39.94 |
| 3 | buried | 30.77 | buried | 30.77 |
| 4 | buried | 27.21 | buried | 27.21 |
| 35 | 1.54 (dd, <i>J</i> =12.8, 10.2 Hz) 2.35 (dd, <i>J</i> =12.8, 8.2 Hz) | 41.31 | 1.54 (dd, <i>J</i> =12.8, 10.2 Hz) 2.35 (dd, <i>J</i> =12.8, 8.2 Hz) | 40.72 |
| 36 | - | 85.93 | - | 86.92 |
| 37 | 1.49 (s) | 27.56 | 1.49 (s) | 27.51 |
| 1' | 3.01 (m) | 36.86 | 3.01 (m) | 36.74 |
| 2' | 2.22 (m) 2.63 (m) | 57.76 | 2.22 (m) 2.63 (m) | 57.94 |
| N-Me | 2.29 (s) | 44.75 | 2.29 (s) | 44.80 |

*¹H-, ¹³C-NMR data not listed are very closed to starting material (**2**).

Fig. 24. Structure of the amine derivative (**5**)

The FAB-MS and HR-FAB-MS deduced the molecular weight and the molecular formula of derivative (**6a**) and (**6b**) to 780 and $C_{45}H_{88}O_6N_4$, respectively. The IR (ν_{max} 1670 cm^{-1}) and 1H , ^{13}C -NMR (Table 6) made clear that **6a** and **6b** had a γ -lactam and no double bond as in **5**. In ^{13}C -NMR spectra, an *N,O*-acetal carbon signals (δ_C 85.93, 86.92) such as in derivative (**5**) disappeared, and a new *N,N*-acetal (aminal) carbon signals (δ_C 75.78, 76.05) appeared. An additional analysis of H-H-COSY, C-H-COSY and HMBC led the structure of **6a** and **6b** to be as shown in Fig. 25. The ratio of existence of **6a** to **6b** was *ca.* 3:1 from the signal intensity of ^{13}C -NMR.

This compound (**6**) decomposed into **5** by an addition of water, and the behavior on the silica gel mentioned above could be explained based on this observation.

Table 6. NMR data of the amine derivative (**6**)

| | 6a (major) | | 6b (minor) | |
|------|-------------------|------------|-------------------|------------|
| | δ_H | δ_C | δ_H | δ_C |
| 1 | - | 177.80 | - | 176.90 |
| 2 | 2.60 (m) | 39.14 | 2.60 (m) | 39.14 |
| 3 | buried | 31.06 | buried | 31.06 |
| 4 | buried | 27.07 | buried | 27.07 |
| 35 | 1.64 (m) | 36.77 | 1.64 (m) | 36.44 |
| | ~2.4 (m) | | ~2.4 (m) | |
| 36 | - | 75.78 | - | 76.05 |
| 37 | 1.42 (s) | 27.41 | 1.34 (s) | 27.36 |
| 1' | 3.02 (m) | 38.51 | 3.02 (m) | 38.09 |
| | ~3.8 (m) | | ~3.8 (m) | |
| 2' | 2.60 (m) | 57.26 | 2.60 (m) | 57.87 |
| | ~2.4 (m) | | ~2.4 (m) | |
| 1'' | 3.09 (m) | 40.46 | 3.09 (m) | 40.79 |
| | ~2.4 (m) | | ~2.4 (m) | |
| 2'' | 2.51 (m) | 59.11 | 2.51 (m) | 58.79 |
| | ~2.4 (m) | | ~2.4 (m) | |
| N-Me | 2.28 (s) | 45.55 | 2.28 (s) | 45.55 |
| | 2.24 (s) | 45.11 | 2.24 (s) | 45.11 |
| | 2.21 (s) | 45.47 | 2.21 (s) | 45.47 |
| | 2.18 (s) | 45.35 | 2.18 (s) | 45.35 |

* 1H -, ^{13}C -NMR data not listed are very closed to starting material (**2**).

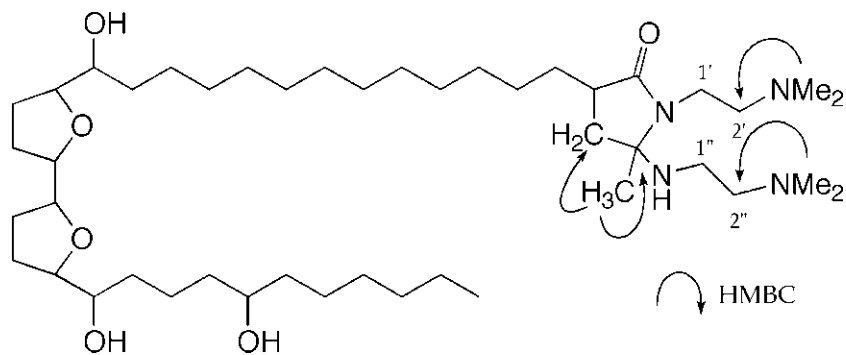


Fig. 25. Structure of the amine derivative (6)

7. Precursor ion scanning of *N,N*-dimethylethylenediamine derivative (5)

The derivatization of natural compounds for MS must satisfy following prerequisites;

- (1) Derivatization can be performed in a trace amount.
- (2) Work-up procedure is not needed or should be easy.
- (3) Several samples can be derivatized at a same time.

From these point of view, my co-worker: Dr. K. Hirayama et al. examined to derivatize squamocin (2) with *N,N*-dimethylethylenediamine at a gas phase (80 °C, 20 hrs).

The reaction mixture was analyzed on a silica gel TLC experiment. The derivative (5) and the starting material (2) (60%) were observed, however, derivative (6) was not detected on the TLC (Derivative (6) was not observed by MS experiment, either). The precursor ion scanning was performed for the derivative (5).

The FAB-MS spectrum of derivative (5) is shown in Fig. 26. Expectedly, fragment ion m/z 72 was detected as an intense peak.

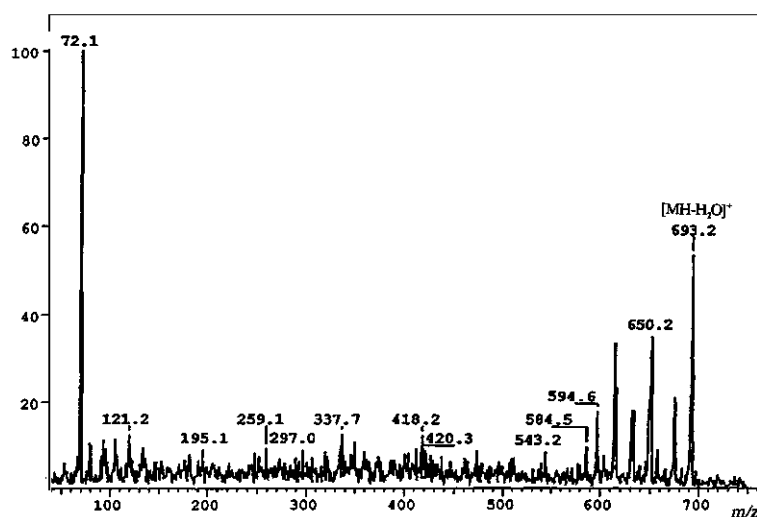


Fig. 26. The FAB-MS spectrum of the amine derivative (5)

Precursor ion scanning of this sample from m/z 72 was performed (Fig. 27), and fragment ions cleaved at alpha position of 28-OH on the b-chain (m/z 625 (C-28/C-29), 607 (C-28/C-29 - H₂O)), which could not be observed in a sufficient S/N ratio by usual measurement mode previously, was clearly detected for the first time.

Therefore, these results demonstrated that the interpretation of the spectra make elucidation of the planar struc-

ture of acetogenin easy and secure, because almost all C-C fission fragment ions were observed.

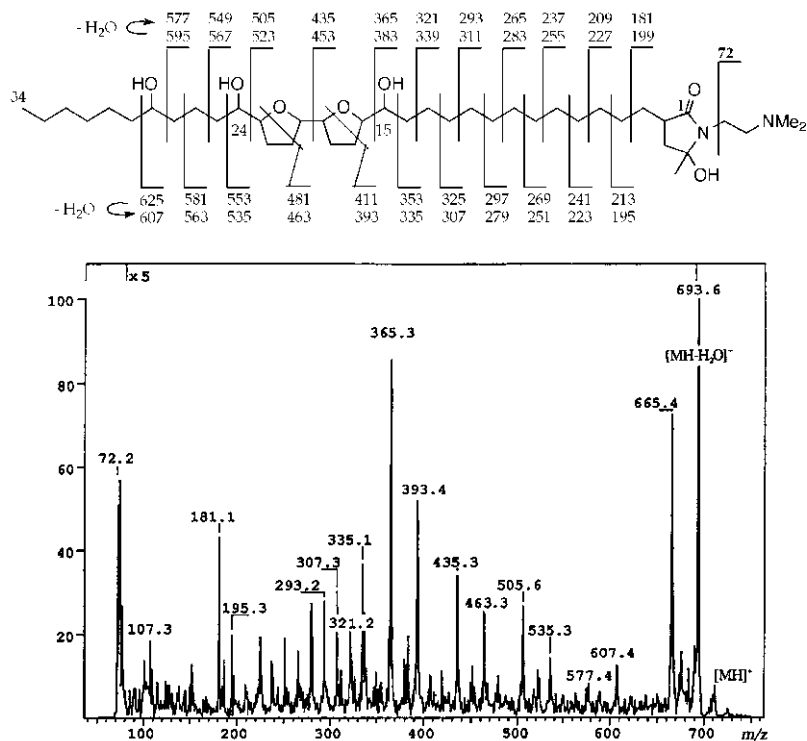
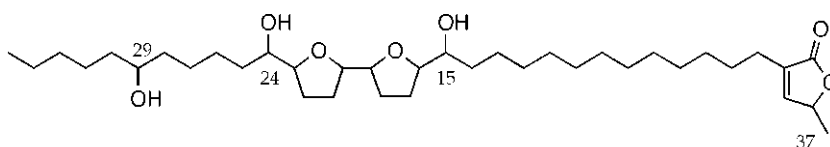


Fig. 27. Precursor ion scanning from m/z 72 for the amine derivative (5)

8. Application of the amine method to other tetrahydrofuranic acetogenins

As mentioned above, combination of both derivatization with *N,N*-dimethylethylenediamine and the precursor ion scanning method (call this method as "amine method") had now been established to squamocin (2). This method was further applied to other acetogenins isolated from *A. squamosa* L. or *A. reticulata* L.; squamocin-C (7), squamocin-F (8) (Sahai et al., 1994) (adjacent bis-THF type acetogenins), squamostatin-C (9) (Fujimoto et al., 1994) (non-adjacent bis-THF acetogenin) and annonacin-10-one (10) (Araya et al., 2004; Xu and Chang, 1989) (mono-THF acetogenin).

(1) Application of the amine method to squamocin-C (7)



Squamocin-C

Squamocin-C (7) (Sahai et al., 1994), an adjacent bis-THF type acetogenin, has a hydroxyl group at C-29 on the b-chain. In the EI-MS experiment, the fragment ions of a fission indicating position of the hydroxyl group, m/z 533 (C-29/C-30, 0.61 %) , 515 (C-29/C-30 - H_2O , 1.11 %), 497 (C-29/C-30 - $2H_2O$, 0.98 %), were detected at low intensities. The amine method was applied to the acetogenin (7) (Fig. 28). In this spectra, diagnostic ions for the fission at C-29 [m/z 639 (C-29/C-30), 621 (C-29/C-30 - H_2O), 609 (C-28/C-29) and 591 (C-28/C-29 - H_2O)] were clearly observed as sufficiently intense signals.

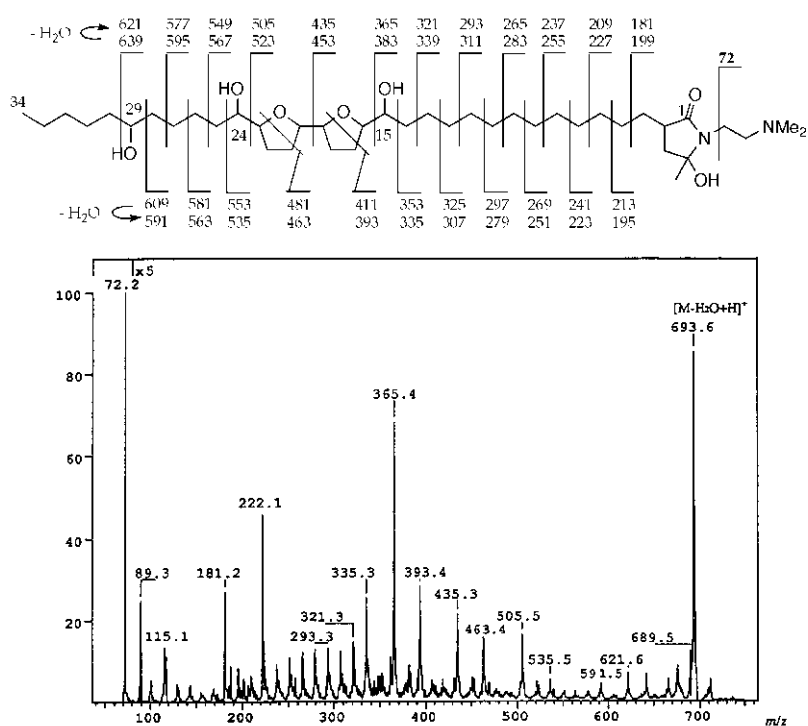
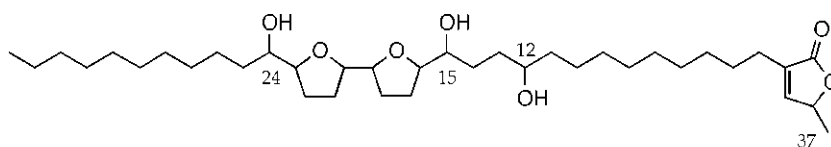


Fig. 28. Precursor ion scanning from m/z 72 for amine derivative of squamocin-C

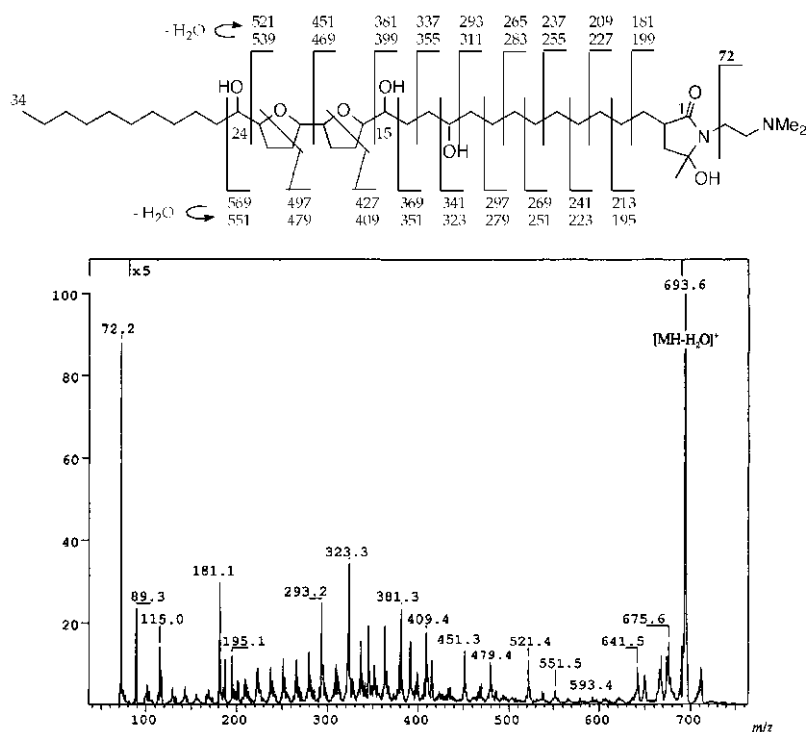
(2) Application of the amine method to squamocin-F (8)



Squamocin-F

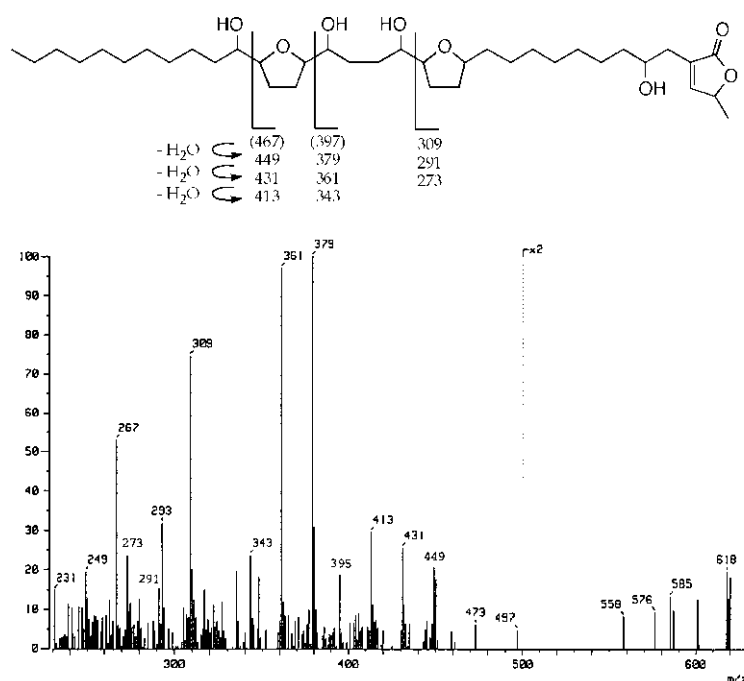
Squamocin-F (**8**) is an acetogenin where hydroxyl group located at C-12. The presence of the hydroxyl group was predicted by combination of NMR and MS experiment, but the direct evidence was not obtained yet. The product ion scan methods from m/z 393 (C-19/C-20 - H_2O) and 293 (C-15/C-16 - H_2O) were also not effective.

The result of the amine method on compound (**8**) is shown in Fig. 29. The occurrence of strong fragment ions at m/z 323 (C-12/C-13 - H_2O), 293 (C-11/C-12 - H_2O) clearly indicated the presence of hydroxyl group at C-12.

Fig. 29. Precursor ion scanning from m/z 72 for amine derivative of squamocin-F

(3) Application of the amine method to squamostatin-C (9)

The position of THF rings of squamostatin-C (**9**) (Fujimoto et al., 1994), a non-adjacent bis THF type acetogenin, could be determined by EI-MS spectra easily, because of its strong fragment ions due to glycol fission (Fig. 30), and the NMR spectral data also supported its structure. However, it is important to clearly distinguish **9** from 4-Hydroxy-squamocin-F, an adjacent bis-THF acetogenins, which is a very close homolog concerning the glycol position (Fig. 31).

Fig. 30. EI-MS spectrum of squamostatin-C (**9**)

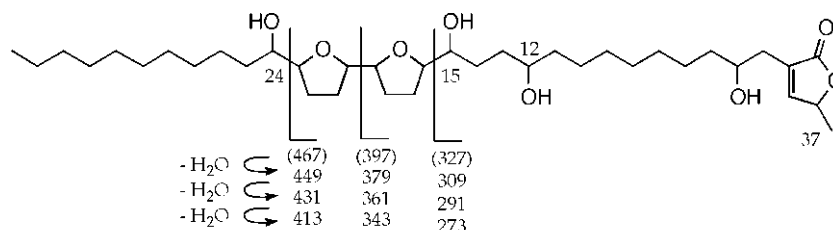
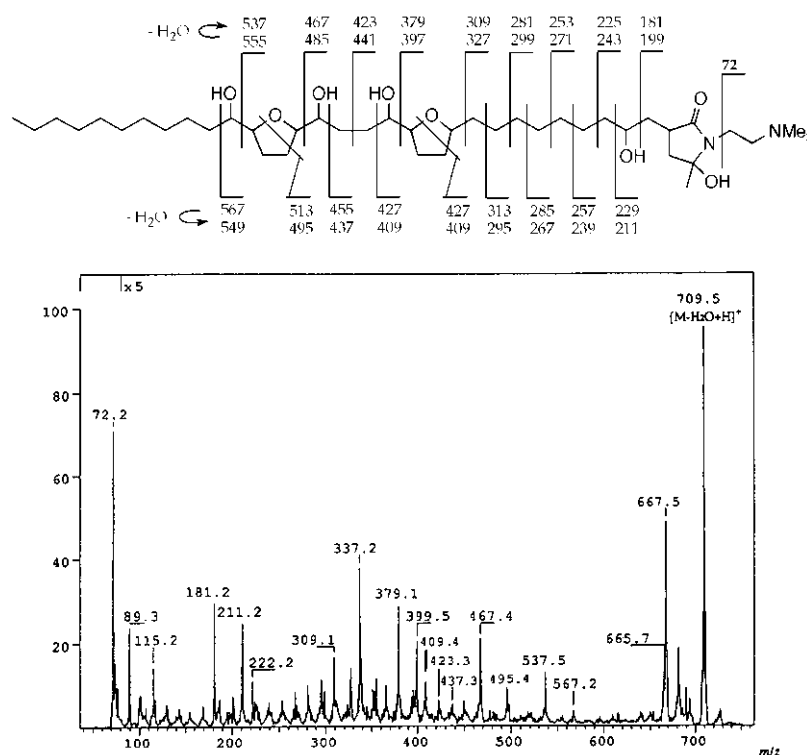


Fig. 31. EI-MS fragment pattern of 4-hydroxy-squamocin-F

Fig. 32 shows the result of the amine method application to squamostatin-C (**9**). Apparently, fragment ions m/z 437 (C-18/C-19 - H₂O), 423 (C-17/C-18 - H₂O) and 409 (C-16/C-17 - H₂O) were detected clearly. This result indicated that the amine method made possible to determine a planar structure of acetogenins accurately.

Fig. 32. Precursor ion scanning from m/z 72 for amine derivative of squamostatin-C

(4) Application of the amine method to annonacin-10-one (**10**)

Annonacin-10-one (**10**) (Araya et al., 2004; Xu and Chang, 1989), possessing one THF ring, was isolated from the seeds of *Annona reticulata* L. Interestingly, this compound has a carbonyl group on the α -chain. Generally, alpha and beta fission of a carbonyl group on the hydrocarbon chain is expected in EI-MS. That is, simple fission at alpha position or at beta position followed by McLafferty rearrangement is observed. However, neither alpha fission nor beta fission was detected clearly for **10** in EI-MS (Fig. 33). In order to determine the position of the carbonyl group by conventional method, the high resolution MS experiment (Xu and Chang, 1989) or a reduction to hydroxyl group (Li et al., 1990; Cortes et al., 1991) is needed.

A result of application of the amine method to **10** was shown in Fig. 34. Fragment ions of fission alpha to the carbonyl group (m/z 309 (C-10/C-11 - H₂O), 281 (C-9/C-10 - H₂O)) were not observed at high intensity. On the other hand, beta fission fragment ions m/z 323 (C-11/C-12 - H₂O), 267 (C-8/C-9 - H₂O) were clearly detected. These fragment ions

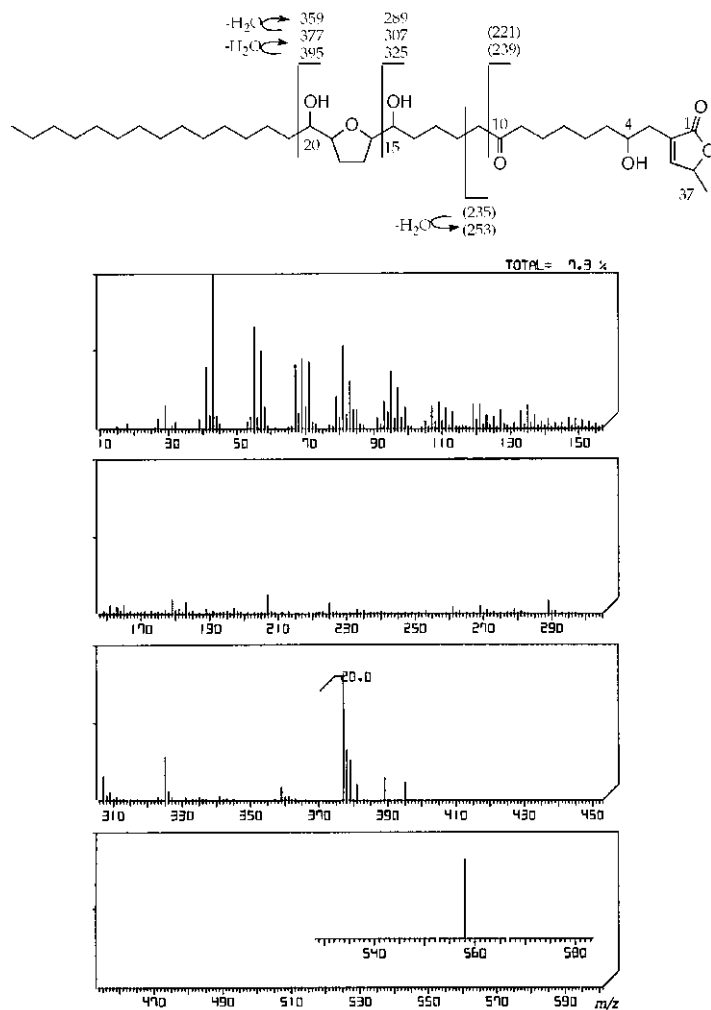


Fig. 33. EI-MS spectrum of annonacin-10-one (10)

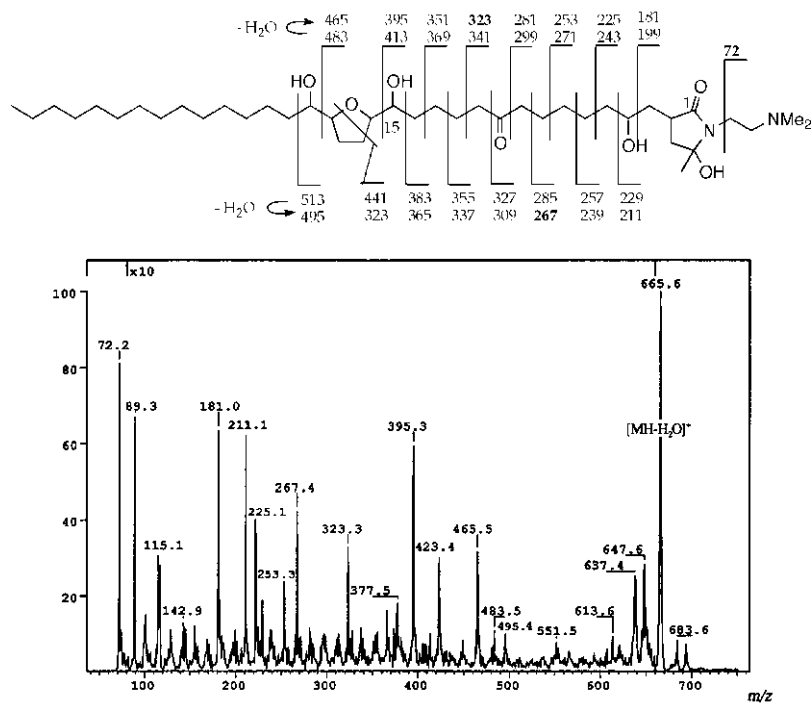


Fig. 34. Precursor ion scanning from m/z 72 for amine derivative of annonacin-10-one

must occur through McLafferty rearrangement and dehydration. A position of the carbonyl group can then be deduced from this feature.

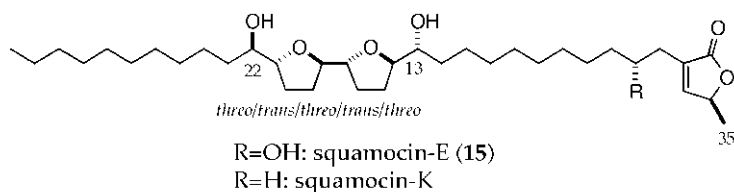
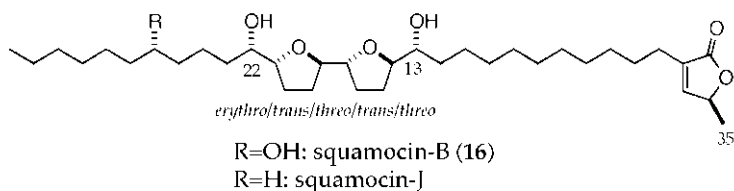
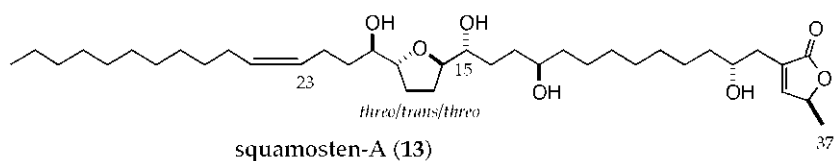
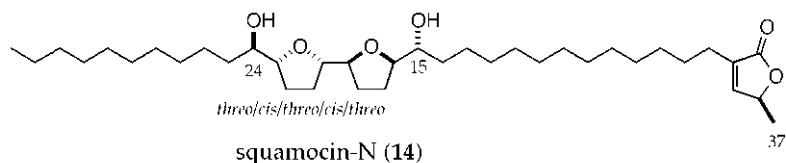
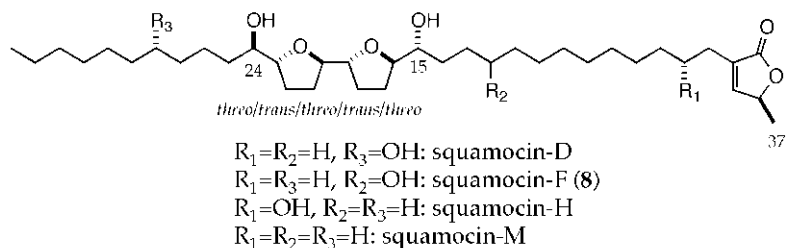
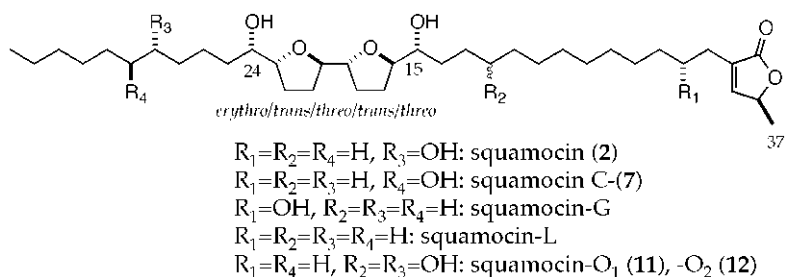
In summary, as mentioned above, it became clear that the amine method, the combination of a derivatization with *N,N*-dimethylethylenediamine and the precursor ion scanning method, is a very effective analytical method for determination of a planar structure of tetrahydrofuranic acetogenins.

Chapter

Structure Elucidation of New Tetrahydrofuranic Acetogenins

1. The isolation procedure

In this study, seven new acetogenins; squamocin-O₁ (**11**), -O₂ (**12**) (Araya et al., 2002), squamosten-A (**13**) (Araya et al., 1994a), squamocin-N (**14**), -E (**15**), -B (**16**) (Sahai et al., 1994) and squamostanal-A (**17**) (Araya et al., 1994b) were isolated from petroleum extracts of *Annona squamosa* L. seeds together with sixteen other known acetogenins, and their structures were elucidated. The isolated acetogenins in this study are shown in Fig. 35.



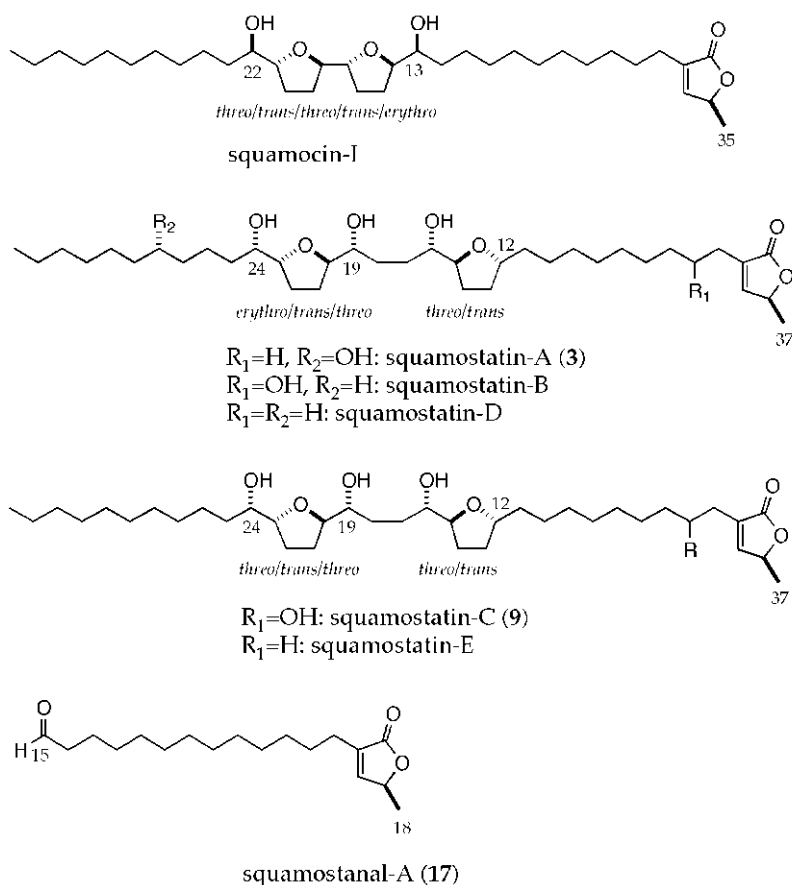


Fig. 35. Isolated tetrahydrofuranic acetogenins in this study

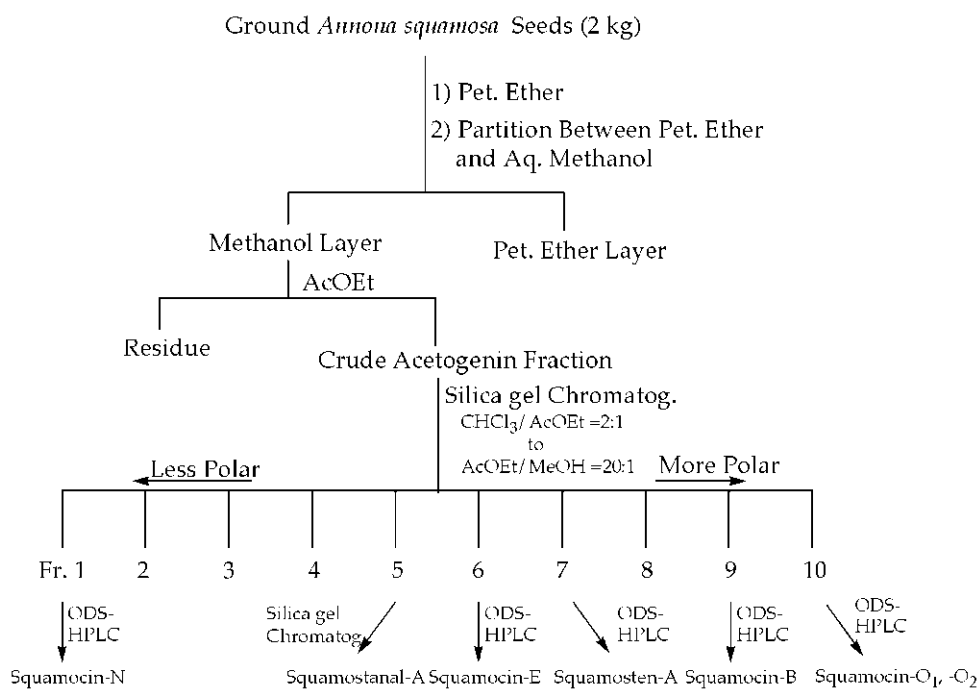


Fig. 36. Isolation of new tetrahydrofuranic acetogenins

The crude acetogenin fraction from seeds of *A. squamosa* L., obtained from India (Dr. Mahendra Sahai, Banaras Hindu University), was chromatographed on silica gel column employing $\text{CHCl}_3/\text{AcOEt}$ and AcOEt/MeOH as eluting solvents. Ten fractions obtained were re-chromatographed on reversed phase HPLC ($\text{MeOH}/\text{H}_2\text{O}$ or $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ as elution solvent). Accordingly, seven novel acetogenins; squamocin- O_1 (**11**) (14 mg), squamocin- O_2 (**12**) (6 mg) (Araya et al., 2002), squamosten-A (**13**) (9 mg) (Araya et al., 1994a), squamocin-N (**14**) (19 mg), squamocin-E (**15**) (54 mg), squamocin-B (**16**) (37 mg) (Sahai et al., 1994) and squamostanal-A (**17**) (2 mg) (Araya et al., 1994b) were obtained (Fig. 36).

2. Structure elucidation of new tetrahydrofuranic acetogenins

(1) Structures of squamocins- O_1 (**11**), - O_2 (**12**)

During the reversed-phase (ODS) HPLC separation, a broad peak eluted prior to squamostatin-A (**3**). NMR analysis of the fraction showed the presence of two compounds. This fraction was separated into two components, named squamocin- O_1 (**11**) (more mobile isomer under the HPLC condition) and squamocin- O_2 (**12**) (less mobile isomer) by a reversed-phase HPLC using methanol-acetonitrile-water-isopropanol (120 : 40 : 30 : 1) as an eluting solvent.

Compounds **11** and **12** showed UV (λ_{max} 210 nm) and IR (ν_{max} 1750 cm^{-1}) absorption typical of α,β -unsaturated- γ -lactone moiety of annonaceous acetogenins. The molecular weight 638 of **11** and **12** was suggested from a pseudo molecular ion peak at m/z 639 in their FAB-MS spectra, and the molecular formula $\text{C}_{37}\text{H}_{66}\text{O}_8$, was deduced from HR-FAB-MS data (found, 639.4792 $[(\text{M}+\text{H})^+]$ and 639.4781 $[(\text{M}+\text{H})^+]$, calcd for $\text{C}_{37}\text{H}_{67}\text{O}_8$, 639.4835). The ^1H - and ^{13}C -NMR spectra of compounds **11** and **12** were very close to each other, and resemble those of squamocin (**2**). The molecular weight of **11** and **12** was larger than that of **2** by 16 mass units, suggesting that compounds **11** and **12** were hydroxylated analogues of **2**. This was proved by the presence of an extra oxymethine signal (δ_{H} 3.8, δ_{C} 72.8) in the NMR spectra of compounds **11** and **12**. Further, the secondary hydroxyl group appears to be located in the methylene chain between C-3 and C-14 on the basis of EI-MS fragmentation pattern (Fig. 37). However, the position of the hydroxyl group could not be assigned from the mass spectrum.

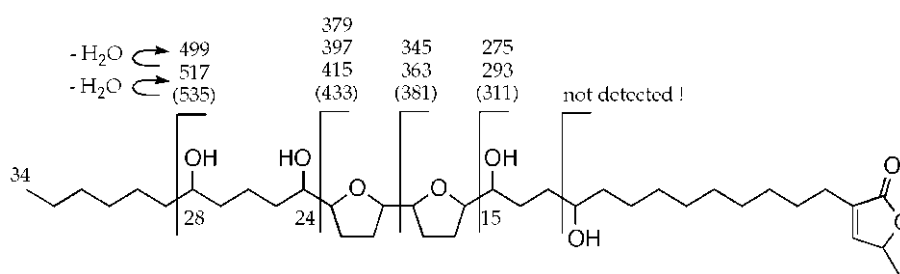
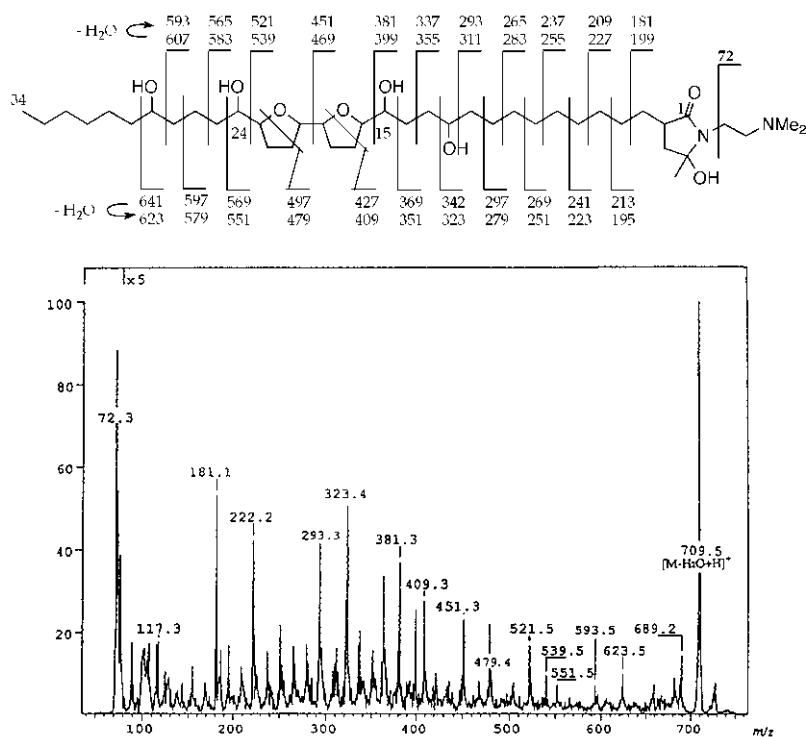


Fig. 37. EI-MS fragment pattern of **11**

The precursor ion scanning application of an acetogenin aminal derivative was described in previous chapter. This method was successfully applied in this case. As can be seen in Fig. 38, the aminal derivative of **11a** (structure was shown in Fig. 38) showed the daughter ions that arose from fission of C11/C12 and C12/C13 followed by dehydration to give m/z 293 and 323, respectively. Thus, binding position of the secondary hydroxyl group was unequivocally assigned to the C-12 position. The spectrum also confirmed the position of two tetrahydrofuran rings and the C-28 hydroxyl group. The precursor ion scanning spectrum of the aminal derivative of **12a** was essentially the same as that of **11a**, indicating that **11** and **12** had the same planar structure. This planar structure was identical to salzmanin, isolated from the roots of *Annona salzmanii* (Queiroz et al., 1999).

Fig. 38. Precursor ion scanning of amina derivative (**11a**) from m/z 72

The relative stereochemistry around tetrahydrofurans of **11** and **12** were easily assigned to *threo/trans/threo/trans/erythro* (from C-15 to C-24) from its ^1H - and ^{13}C -NMR (Table 7) data on the basis of accumulated ^{13}C -NMR data on a number of bis-tetrahydrofuran subclass acetogenins. On the other hand, salzmanin has *threo/trans/erythro/cis/erythro* configuration. Therefore, squamocins- O_1 (**11**) and - O_2 (**12**) were discovered to be new acetogenins.

Table 7. ^{13}C -NMR spectral data for squamocins- O_1 (**11**) and - O_2 (**12**)

| C | 11 | 12 | C | 11 | 12 |
|-----|-----------|-----------|----|-----------|-----------|
| 1 | 173.9 | 173.9 | 22 | 24.8 | 24.8 |
| 2 | 134.3 | 134.3 | 23 | 82.8 | 82.9 |
| 3 | 25.1 | 25.1 | 24 | 71.3 | 71.2 |
| 4 | 27.3 | 27.4 | 25 | 32.4 | 32.5 |
| 5-9 | a | a | 26 | 22.1 | 22.2 |
| 10 | 25.8 | 25.8 | 27 | 37.3 | 37.4 |
| 11 | 37.5 | 37.7 | 28 | 71.7 | 71.9 |
| 12 | 71.5 | 71.7 | 29 | 37.5 | 37.7 |
| 13 | 33.5 | 34.3 | 30 | 25.6 | 25.7 |
| 14 | a | a | 31 | a | a |
| 15 | 74.3 | 74.6 | 32 | 31.8 | 31.9 |
| 16 | 83.1 | 83.2 | 33 | 22.6 | 22.6 |
| 17 | 28.4 | 28.4 | 34 | 14.0 | 14.1 |
| 18 | 28.9 | 29.0 | 35 | 148.9 | 148.8 |
| 19 | 82.2b | 82.2c | 36 | 77.4 | 77.4 |
| 20 | 82.5b | 82.5c | 37 | 19.2 | 19.2 |
| 21 | 28.9 | 29.0 | | | |

a) The signals appeared in the region of δ 29.0-30.0.

b-c) Assignments may be interchanged within the column.

The next step is to determine the absolute configuration of their stereogenic centers. First, the CD spectra of **11** and **12** showed a negative Cotton effect at 239 nm, thus the absolute stereochemistry at C-36 was determined as *S*, as is common to all other reported annonaceous acetogenins (Sahai et al., 1994).

Next, compounds **11** and **12** were converted into their tetra-(*R*)-MTPA ester (**11r** and **12r**) and tetra-(*S*)-MTPA ester (**11s** and **12s**) respectively to obtain further information on the other stereogenic carbinol centers. Their ¹H-NMR spectral data were carefully compared with those of the squamocin MTPA esters (**2r** and **2s**) (Table 8). The C-28 configuration of compounds **11** and **12** were deduced from the chemical shifts of terminal methyl group (C-34). McLaughlin's group (Gu et al., 1994b) and our group (Nishioka et al., 1994) have previously reported the utility of this chemical shift, which depended on the configuration at C-28. The (*R*)-MTPA esters **11r**, **12r** and **2r** all displayed the

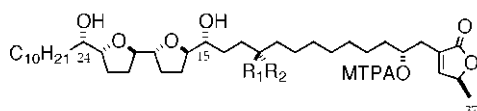
Table 8. ¹H-NMR spectral data for MTPA esters (**11rs**, **12rs** and **2rs**)

| C | 11r | 12r | 2r |
|-----|----------------|----------------|----------------|
| 4 | 2.267 (t) | 2.265 (t) | 2.264 |
| 12 | 4.94 (m) | 5.02 (m) | - |
| 15 | 4.98 (m) | 4.95 (m) | 5.02 (m) |
| 16 | 3.96 (q) | 3.89 (m) | 3.99 (m) |
| 19 | 3.63 (m) | 3.62 (m) | 3.65 (m) |
| 20 | 3.80 (m) | 3.78 (m) | 3.83 (m) |
| 23 | 3.86 (m) | 3.86 (m) | 3.87 (m) |
| 24 | 5.14 (q) | 5.14 (m) | 5.14 (m) |
| 28 | 5.02 (m) | 5.02 (m) | 5.02 (m) |
| 34 | 0.874 (t, 7.2) | 0.874 (t, 7.2) | 0.874 (t, 7.2) |
| 35 | 6.98 (br s) | 6.98 (br s) | 6.98 (br s) |
| 36 | 4.99 (m) | 4.99 (m) | 4.99 (m) |
| 37 | 1.40 (d, 6.4) | 1.39 (d, 6.4) | 1.41 (d, 6.4) |
| MeO | 3.484 | 3.509 | 3.513 |
| | 3.514 | 3.509 | 3.531 |
| | 3.531 | 3.528 | 3.609 |
| | 3.595 | 3.565 | |

| C | 11s | 12s | 2s |
|-----|----------------|----------------|----------------|
| 4 | 2.260 (t) | 2.259 (t) | 2.263 (t) |
| 12 | 5.00 (m) | 5.09 (m) | - |
| 15 | 5.00 (m) | 5.09 (m) | 5.06 (q) |
| 16 | 3.94 (m) | 3.99 (m) | 4.03 (q) |
| 19 | 3.77 (m) | 3.78 (m) | 3.79 (m) |
| 20 | 3.77 (m) | 3.78 (m) | 3.79 (m) |
| 23 | 3.94 (m) | 3.95 (m) | 3.96 (m) |
| 24 | 5.20 (q) | 5.20 (m) | 5.20 (q) |
| 28 | 5.00 (m) | 4.99 (m) | 4.99 (m) |
| 34 | 0.859 (t, 7.2) | 0.860 (t, 7.2) | 0.860 (t, 7.2) |
| 35 | 6.98 (br s) | 6.98 (br s) | 6.98 (br s) |
| 36 | 4.99 (m) | 4.99 (m) | 4.99 (m) |
| 37 | 1.40 (d, 6.4) | 1.39 (d, 6.4) | 1.40 (d, 6.4) |
| MeO | 3.488 | 3.504 | 3.510 |
| | 3.505 | 3.504 | 3.534 |
| | 3.531 | 3.515 | 3.546 |
| | 3.531 | 3.532 | |

Table 9. Partial NMR $\Delta\delta$ value of MTPA esters (**11r**, **12r**, **18r** and **19r**)

| esters | H-12 | H-15 | H-16 | H-19 | H-20 |
|--------------------------------------------------|------|-------|-------|-------|-------|
| 11r | 4.94 | 4.98 | 3.96 | 3.63 | 3.80 |
| 12r | 5.02 | 4.95 | 3.89 | 3.62 | 3.78 |
| 18r | 4.93 | 4.97 | 3.96 | 3.63 | 3.80 |
| 19r | 5.00 | 4.95 | 3.89 | 3.62 | 3.79 |
| $\Delta\delta_{\text{H}(11\text{r}-12\text{r})}$ | - | +0.03 | +0.07 | +0.01 | +0.02 |
| $\Delta\delta_{\text{H}(18\text{r}-19\text{r})}$ | - | +0.02 | +0.07 | +0.01 | +0.01 |



$R_1 = \text{O}-(R)\text{-MTPA}$, $R_2 = \text{H}$: **18r**
 $R_1 = \text{H}$, $R_2 = \text{O}-(R)\text{-MTPA}$: **19r**

34-methyl at δ 0.874, while the (*S*)-MTPA esters **11s**, **12s** and **2s** all displayed the 34-methyl at δ 0.860. Since the C-28 configuration of **2** was established to be *S*, compounds **11** and **12** must have the same configuration at C-28.

Compounds **11** and **12** were same *threo/trans/threo/trans/erythro* configuration around tetrahydrofuran moiety as mentioned above. That is, two absolute configurations, 15*R*,16*R*,19*R*,20*R*,23*R*,24*S* and 15*S*,16*S*,19*S*,20*S*,23*S*,24*R* are possible for this portion. Squamocin (**2**) is established to have the former absolute stereochemistry (Araya et al., 1994a; Araya et al., 1994c). Comparison of the ¹H-NMR data shown in Table 8 reveals that the chemical shifts of 28-H, 24-H and 23-H for (*R*)- and (*S*)-MTPA esters (**11rs** and **12rs**) are identical to those of **2rs**. These facts indicate that the tetrahydrofuran moiety of **11** and **12** has the same configuration as in **2**. Therefore, the absolute stereochemistry of the tetrahydrofuran moiety of **11** and **12** were assigned as 15*R*,16*R*,19*R*,20*R*,23*R*,24*S*. The signals of H-19 and H-20 appeared almost at the same chemical shifts, depending on the (*R*)- or (*S*)-MTPA ester. In contrast, the chemical shifts at H-15 and H-16 of **11rs**, **12rs** and **2rs**, were not simply classified into two categories. This seems to be the anisotropic effect of the C-12 MTPA ester group. Application of the advanced Mosher method appears to be difficult in this portion (C-12 to C-16) because of the presence of multiple MTPA groups in the molecule and the close 1,4-diol relationship. Recently, McLaughlin's group determined the absolute stereochemistry of carbinol centers in 12-hydroxy-bullatacins A (**18**) and B (**19**) by NMR analysis of its cyclic formaldehyde acetals (Shi, et al., 1997b). They utilized MTPA esters of **18** and **19**, together with four other known epimeric pairs of acetogenins, and proposed this novel application to epimeric carbinols for determination of absolute stereochemistry. This method was applied for the determination of absolute configuration at C-12 of **11** and **12**. The NMR data of MTPA esters of **11r** and **12r** were compared to those of 12-hydroxy-bullatacins A (**18r**) and B (**19r**) (Table 9). As the sign of $\Delta\delta_{\text{H}(2r-3r)}$ was identical to that of $\Delta\delta_{\text{H}(4r-5r)}$, C-12 of **11** and **12** were concluded to have the respective *R* and *S* configurations, respectively. This result was in agreement of the ¹³C-NMR chemical shifts at C-12 and C-15 (δ_{C} **11**: 71.5, 74.3; **12**: 71.7, 74.6; 12-hydroxy-bullatacin A: 71.5, 74.2; 12-hydroxy-bullatacin B: 71.8, 74.4). Thus, structures of squamocins-O₁ (**11**) and -O₂ (**12**) were determined as shown in Fig. 39.

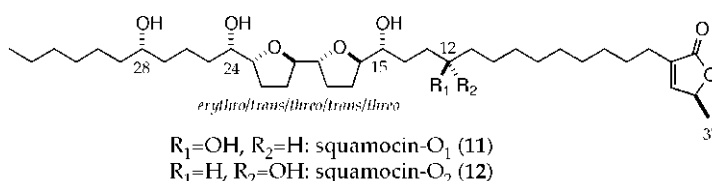


Fig. 39. Chemical structures of squamocins-O₁ (**11**) and -O₂ (**12**)

(2) Structure of squamosten-A (**13**)

The physico-chemical property of squamosten-A (**13**) is shown in Table 10. The IR, UV, ¹H-NMR (δ 7.18 (H-35), 5.05 (H-36), 1.39 (H-37)) and ¹³C-NMR (δ 174.58 (C-1), 151.78 (C-35), 131.24 (C-2), 77.96 (C-36), 19.12 (C-37)) spectra indicate that this compound contains characteristic α,β -unsaturated- γ -lactone of tetrahydrofuranic acetogenins. Further,

Table 10. Physico-chemical properties of squamosten-A (**13**)

| | |
|------------------------------------------|----------------------------------------------------------------------------------------------------------|
| State | White solid, mp 64-67 °C |
| M.W. | 622 (FAB-MS: MH ⁻ 623) |
| M.F. | C ₃₇ H ₆₆ O ₇ (HR-FAB-MS: 623.4844 (MH ⁺ , Calcd. 623.4887)) |
| [α] _D ²⁵ | +9.0 (c=0.10, MeOH) |
| UV (λ_{max} ϵ) | 210 nm (7000) |
| IR (ν_{max}) | 3665, 3575, 3450, 1745 (cm ⁻¹ , CHCl ₃) |
| CD | $\Delta\epsilon$ -0.57 (MeOH, at 240 nm) |

methylene protons (δ 2.53, 2.40) at C3 are coupled in an ABX type pattern, and ^{13}C -NMR chemical shift at C-3, -4 and -5 (δ 33.38, 69.99, 37.39) were in accordance with known compounds (Sahai et al., 1994) with a hydroxyl group at C-4. These facts indicated that compound (**13**) has a (a') type lactone.

NMR data (oxymethylene protons; ^1H -NMR: δ 3.44 (2H), 3.83 (2H), and carbons; ^{13}C -NMR: δ 73.50, 74.37, 82.63, 83.65) indicated that **13** has (C1) type core. This fact was supported by Born's rule (Table 3) and an indication of existence of two $-\text{C}-\text{CH}_2\text{OMTPA}-\text{CH}_2\text{OR}-\text{C}-$ structures (H_a and H_b are coupled) in ^1H -NMR of its (*R*)-MTPA ester. The NMR data indicate the presence of two more secondary hydroxyl groups (δ_{H} 3.63, δ_{C} 71.70) and a double bond (δ_{H} 5.36, 5.39, δ_{C} 128.92, 130.87).

In the EI-MS spectra, fragment ions, m/z 309 (C-15/C-16 - H_2O), 379 (C-19/C-20 - H_2O) made clear that a tetrahydrofuran was located at C-16/C-19 and a hydroxyl group and a double bond were located on different chains to each other (Fig. 40). In this spectrum, a fragment ion m/z 269 (C-12/C-13) indicated that existence of 12-OH, but its dehydrogenated ion m/z 251 (C-12/C-13 - H_2O) was not detected. Additionally, fragment ions m/z 327 (C-15/C-16) and 397 (C-19/C-20), which indicate the presence of hydroxyl group on the a-chain, were not detected. As a result, the position of 12-OH could not be established by analysis of NMR and MS data.

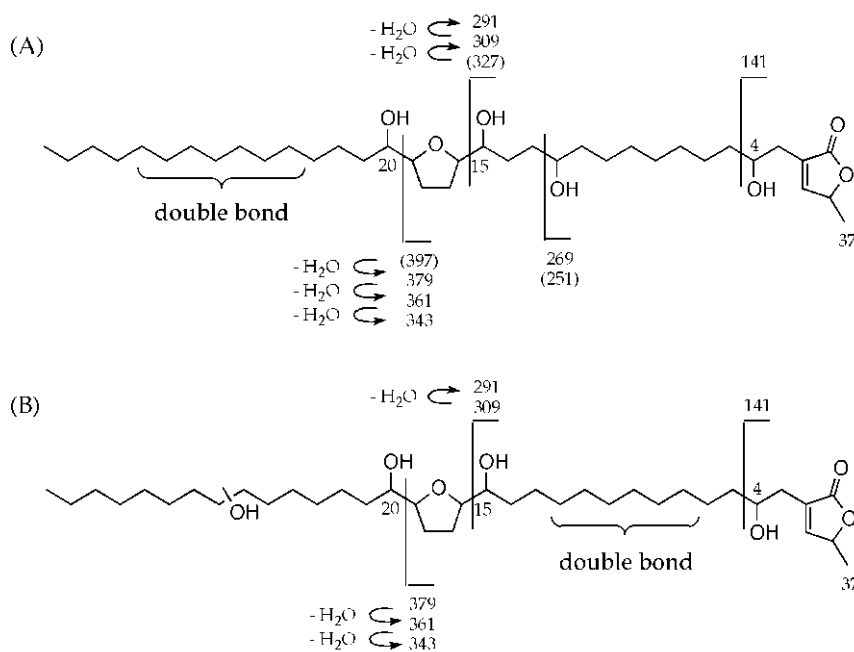
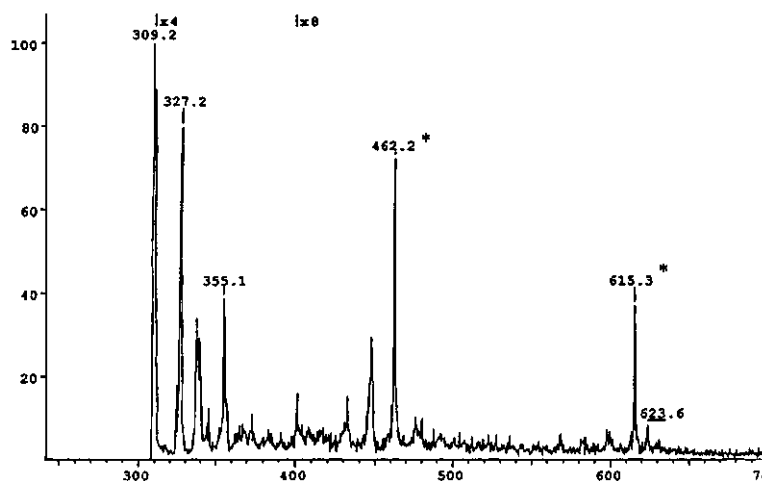


Fig. 40. Possible structure for squamosten-A (**13**)

From above data, compound **13** must be focused to two planar structures (A) and (B) depicted in Fig. 40. When m/z 327 fragment ion is observed by the precursor ion scanning from m/z 309, this compound could be determined as (A). Precursor ion scanning from m/z 309 was showed in Fig. 41. The fragment ion m/z 327 was observed clearly, thus compound **13** was determined to have the planar structure (A).

A detailed NMR study was carried out to confirm the position of 12-OH group, from its 1-4 relationship to 15-OH. This was supported by ^{13}C -NMR as well (chemical shift of C-13, overlapped methylene peaks (δ_{C} ca. 29)). When the amine method was applied to this compound, diagnostic ions of a fission of 12-OH, m/z 339 (C-12/C-13 - H_2O), 309 (C-11/C-12 - H_2O) were observed clearly.

In order to determine the position of the double bond on the b-chain, compound **13** (55 μg) was treated with ruthenium oxidation (ruthenium trichloride, sodium periodate) (Carlsen et al., 1981), and the resulting fatty acid was



* m/z 462 and m/z 615 ions were added one or two molecular of *m*-nitrobenzyl alcohol as a matrix to ion m/z 309 respectively.

Fig. 41. Precursor ion scanning from m/z 309 for squamosten-A (**13**)

derivatized to *p*-bromophenacyl ester (Tokyo Kasei Kogyo). The resulting ester was compared with authentic esters by reversed phase HPLC. It was obvious as shown in Fig. 42 that the fatty acid derived from **13** was undecanoic acid. This result indicated the double bond was situated on C-23/C-24. The coupling constant of 11.0 Hz ($J_{H-23,H-24}$) obtained by 1H -NMR decoupling experiment (H-22 and H-24 were irradiated) confirmed the *cis* configuration of the double bond.

Subsequently, the stereochemistry of compound **13** was investigated. The relative stereochemistry of both C-15/C-16 and C-19/C-20 were determined to be *threo* by the Born's rule (Table 3). The tetrahydrofuran was determined to be *trans* by comparison of 1H -NMR, ^{13}C -NMR with model compounds (the result of ^{13}C -NMR was shown in Table 11) (Fujimoto et al., 1994).

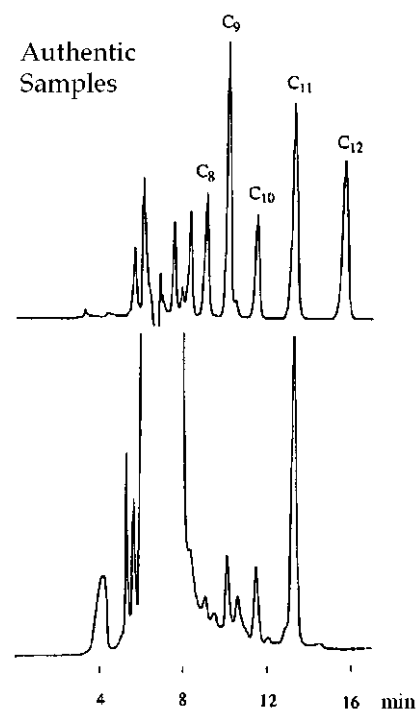
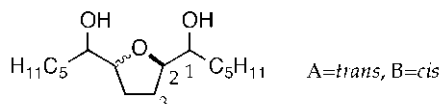


Fig. 42. HPLC analysis of fatty acid *p*-bromophenacyl ester

Table 11. ^{13}C -NMR comparison of the signals of tetrahydrofuran moiety

| Compound | (C-15, -20)/(C-1) | (C-16, -19)/(C-2) | (C-17, -18)/(C-3) |
|--------------------|-------------------|-------------------|-------------------|
| Squamosten-A | 74.37/73.50 | 82.63/83.65 | 28.74/28.71 |
| A (<i>trans</i>) | 74.04 | 82.73 | 28.76 |
| B (<i>cis</i>) | 74.32 | 82.76 | 28.07 |



The absolute stereochemistry of 4-OH of compound **13** was determined by comparison of its (*R*)-MTPA ester with MTPA esters of squamocin-G. That is, squamocin-G (=bullatacin, rolliniastatin-2) was derivatized to (*R*)- and (*S*)-MTPA ester, and determined the 4-OH to *R* configuration by the advanced Mosher method (Ohtani et al., 1991). (Table 12, Fig. 43). (*R*)-MTPA ester of **13** closely resembled to (*R*)-MTPA ester of squamocin-G, the carbinol center at C-4 of **13** was thus determined to have *R* stereochemistry.

Table 12. ¹H-NMR data of (*R*)- and (*S*)-MTPA ester of squamocin-G and (*R*)-MTPA ester of squamosten-A

| | 3-Ha | 3-Hb | H-4 | 35-H | 36-H | 37-H | 5-H _a ,H _b ^{a)} |
|--------------------------------------|-------|------|-------|-------|-------|-------|------------------------------------------------|
| (<i>R</i>)-ester of Sq-G | 2.59 | 2.68 | 5.38 | 6.97 | 4.9 | 1.31 | 1.56, 1.65 |
| (<i>S</i>)-ester of Sq-G | 2.58 | 2.58 | 5.32 | 6.73 | 4.86 | 1.28 | 1.63, 1.69 |
| $\Delta\delta_{(S) \rightarrow (R)}$ | -0.01 | -0.1 | -0.06 | -0.24 | -0.04 | -0.03 | 0.11 |
| (<i>R</i>)-ester of (13) | 2.6 | 2.68 | 5.37 | 6.96 | 4.91 | 1.31 | 1.57, 1.66 |

a) These signals were assigned on the basis of ¹H-¹H-COSY experiment.

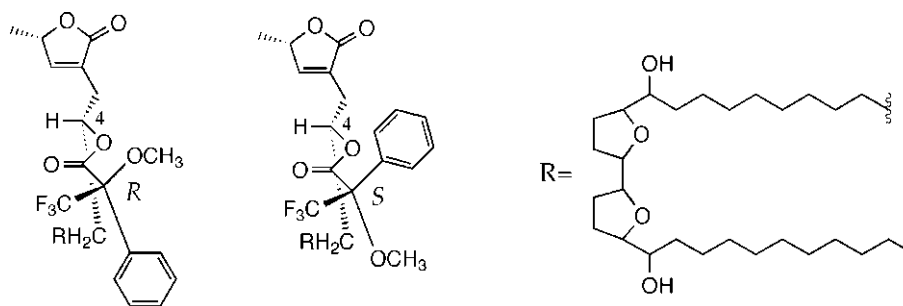
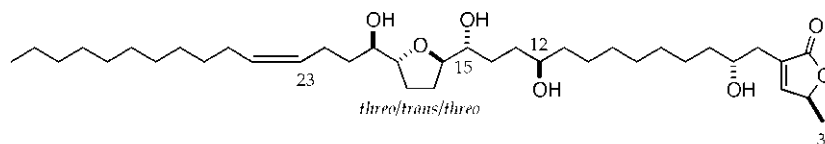


Fig. 43. Mosher's model for (*R*)- and (*S*)-MTPA esters

The absolute stereochemistry of C-36 was determined to be *S*, because of a negative Cotton effect observed at 240 nm on CD spectra similar to that of squamocin (**2**) which was determined to be 36*S* with degradation reaction (Sahai et al., 1994).

The absolute stereochemistry at C-12 and -15 were determined by its comparison with MTPA derivatives (**11r**), (**12r**), (**18r**) and (**19r**) (Table 9). Since proton chemical shifts of **13r** at C-12, C-15 and C-16 resembled to **11r** and **18r**, the absolute stereochemistry of **13** at C-12, -15, -16, -19 and -20 was deduced to be 12*R*,15*R*,16*R*,19*R*,20*R*.

Accordingly, the structure of squamosten-A (**13**) was assigned the following structure.



Squamosten-A

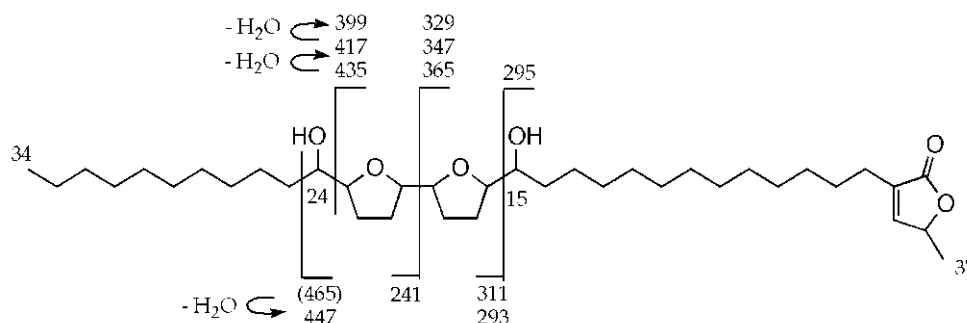
(3) Structure of squamocin-N (**14**)

Squamocin-N (**14**) was the least polar acetogenin in this study. The physico-chemical property of **14** is shown in Table 13. The presence of α,β -unsaturated- γ -lactone was confirmed by IR, UV, ¹H-NMR (δ 6.98 (H-37), 4.99 (H-36), 2.26 (H-3), 1.41 (H-37)) and ¹³C-NMR (δ 173.82 (C-1), 148.86 (C-35), 134.16 (C-2), 77.42 (C-36), 19.23 (C-37)) same as that of squamosten-A (**13**). The lactone portion of **14** was (a) type lactone lacking 4-OH, because methylene protons were observed at δ 2.26 as triplet ($J=7.7$ Hz) in ¹H-NMR.

Table 13. Physico-chemical properties of squamocin-N (**14**)

| | |
|--------------------------------|-------------------------------------------------------------------------------------------------------|
| State | White wax |
| M.W. | 606 (FAB-MS: MH ⁻ 607) |
| M.F. | C ₃₇ H ₆₆ O ₆ (Anal. C, 73.01; H, 11.25 (Calcd. C, 73.22; H, 10.96)) |
| [α] _D ²⁵ | +40.6 (c=0.43, MeOH) |
| UV (λ _{max} , ε) | 210 nm (7000) |
| IR (ν _{max}) | 3560, 3450, 1750 (cm ⁻¹ , CHCl ₃) |
| CD | Δε -0.57 (MeOH, at 240 nm) |

Further, since oxymethyne protons (δ 3.40 (2H), 3.81-3.85 (2H), 3.88-3.93 (2H)) and carbons (δ 74.05 (2C), 81.74 (2C), 82.77 (2C)) were observed in NMR experiments, **14** had two tetrahydrofurans and two hydroxyls belonging to the (A) type acetogenin, which are symmetrical about tetrahydrofuranic portion. The presence of two hydroxyl groups were supported by diagnostic ions (m/z 606 (M⁺), 588 (M⁺ - H₂O), 570 (M⁺ - 2H₂O)) in the EI-MS. The position of bis-tetrahydrofurans was determined to be C-16/C-23 on the basis of the EI-MS (Fig. 44).

Fig. 44. EI-MS fragmentation pattern of squamocin-N (**14**)

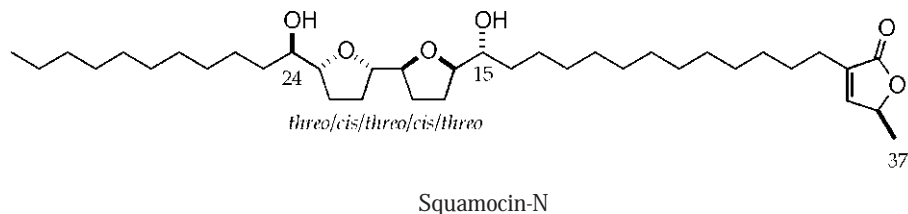
The relative stereochemistry of the bis-tetrahydrofurans portion was determined to be *threo/cis/threo/cis/threo* by the Hoyer's rule (Table 2). That is, ¹H-NMR data of squamocin-N acetate (δ 2.070 (AcO), 3.87 (H-19, -20), 3.94 (H-16, -23), 4.94 (H-15, -24)) was compared with Hoyer's model compounds, and the result was in accordance with *threo/cis/threo/cis/threo* stereochemistry of the model data. The absolute stereochemistry of **14** was determined to be 15*R*,16*R*,19*S*,20*S*,23*R*,24*R* by the advanced Mosher method (Ohtani et al., 1991) as shown in Table **14**. Unexpected chemical shifts of H-16 and -23 were due to low deshielding effects, because of a steric hindrance. The absolute stereochemistry of C-36 was determined as *S* by the CD spectra same as **13**.

Table 14. ¹H-NMR data of squamocin-N (*R*)- and (*S*)-MTPA ester

| | H _{a,b} -14, -25 ^{a)} | H-16, -23 | H-19, 20 |
|-------------------------------------|-----------------------------------------|-----------|----------|
| (<i>R</i>)-ester | 1.49-1.54 | 3.9 | 3.9 |
| (<i>S</i>)-ester | 1.63-1.66 | 3.95 | 3.76 |
| Δδ _{(<i>S</i>)-(<i>R</i>)} | 0.13 | 0.05 | -0.14 |
| presumed sign | + | - | - |

^{a)} These signals were assigned on the basis of ¹H-¹H-COSY experiments.

From above data, squamocin-N (**14**) was assigned the following structure, and it has novel 15*R*,16*R*,19*S*,20*S*,23*R*,24*R*,36*S* absolute configuration.



(4) Structure of squamocin-E (15)

Physico-chemical property of squamocin-E (**15**) is shown in Table 15. The ^1H - and ^{13}C -NMR revealed that **15** has a (a') type γ -lactone accompanying 4-OH. The MS spectral data indicated that **15** was a rare C35 acetogenin.

Table 15. Physico-chemical properties of squamocin-E (**15**)

| | |
|-------------------------------------------------|-------------------------------------------------------------------------------------------------|
| State | White solid, mp. 48-50 °C |
| M.W. | 594 (FAB-MS: MH^- 595) |
| M.F. | $\text{C}_{35}\text{H}_{62}\text{O}_7$ (HR-FAB-MS: 595.4592 (MH^-), Calcd. 545.4574) |
| $[\alpha]_D^{25}$ | +20.9 ($c=0.25$, MeOH) |
| UV (λ_{max} , $\log \epsilon$) | 209 nm (3.7) |
| IR (ν_{max}) | 3665, 3570, 3450, 1745 (cm^{-1} , CHCl_3) |
| CD | $\Delta \epsilon$ -0.40 (MeOH, at 240 nm) |

The tetrahydrofuran moiety of **15** was determined to be type (A) bis-tetrahydrofurans same as that of **14**, because of the presence of oxymethyne protons (δ 3.39 (2H), 3.82-3.90 (~ 4H)), oxymethyne carbons (δ 73.98, 74.02, 81.73 (2C), 83.11 (2C)) observed in NMR experiments. These three hydroxyls were supported with a series of diagnostic ions m/z 594 (M^+), 576 ($\text{M}^+ - \text{H}_2\text{O}$), 558 ($\text{M}^+ - 2\text{H}_2\text{O}$), 540 ($\text{M}^+ - 3\text{H}_2\text{O}$) in the EI-MS too. The position of tetrahydrofuran portion was determined as Fig. 45 from the EI-MS fragment pattern.

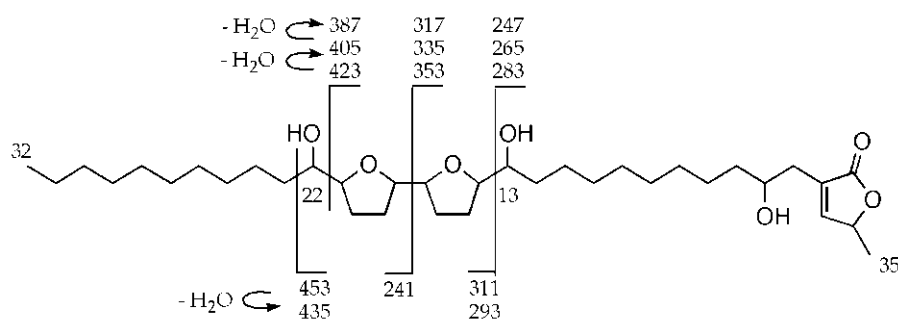


Fig. 45. EI-MS fragmentation pattern of squamocin-E (**15**)

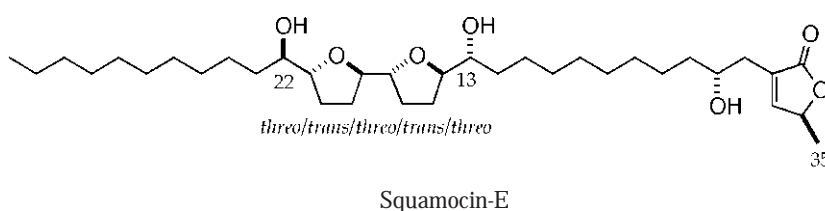
The relative stereochemistry of the tetrahydrofuran portion was easily determined to be *threo/trans/threo/trans/threo* configuration by the comparison with NMR data of known compounds: for example, squamocin (**2**). The absolute stereochemistry of this portion was determined as 13*R*,14*R*,17*R*,18*R*,21*R*,22*R* by comparison of Hoye's squamocin-H (=asimicin) (Rieser et al., 1992) data using advanced Mosher Method (Table 16) (Ohtani et al., 1991). Its 4-OH was determined to be *R* configuration same as in **13**. The absolute stereochemistry of the asymmetric carbon at 34 was determined to be *S* by the CD spectra. The absolute stereochemistry of compound **15** was thus assigned as 4*R*,13*R*,14*R*,17*R*,18*R*,21*R*,22*R*,34*S* configuration as shown below having 35 carbons.

Table 16. ¹H-NMR data of (*R*)- and (*S*)-MTPA esters of squamocin-H and (*R*)-MTPA ester of squamocin-E

| | H-14,-25 ^{a)} | H-16,-23 | H-19,-20 |
|----------------------------|------------------------|----------|----------|
| (<i>R</i>)-ester of Sq-H | 1.45 | 3.97 | 3.92 |
| (<i>S</i>)-ester of Sq-H | 1.56 | 3.93 | 3.76 |
| $\Delta\delta_{(S)-(R)}$ | 0.11 | -0.04 | -0.16 |
| | H-12,-23 ^{a)} | H-14,-21 | H-17,-18 |
| (<i>R</i>)-ester of Sq-E | 1.46 | 4 | 3.93 |

^{a)} These signals were assigned on the basis of ¹H-¹H-COSY experiments.

This compound **15** was a rare (A) type acetogenin having a carbon number 35. The NMR spectra (¹H and ¹³C) could not be distinguished with squamocin-H (=asimicin, molecular weight: 622, molecular formula: C₃₇H₆₆O₇).



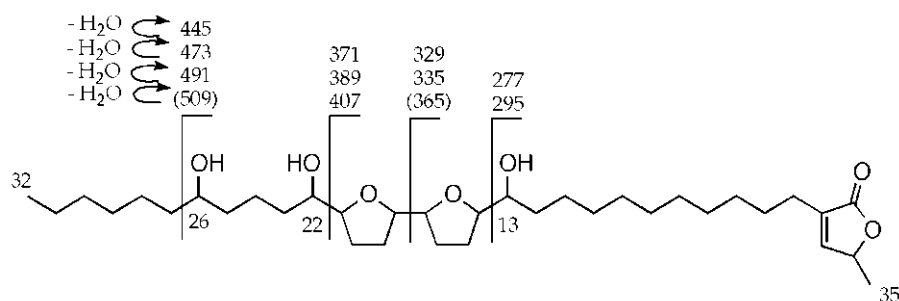
(5) Structure of squamocin-B (16)

The physico-chemical property of squamocin-B is shown in Table 17. Besides the marked lactone portion signals, oxymethyne proton signals (δ 3.38, 3.60, 3.78-3.89 (3H), 3.89-3.93 (2H)) and carbon signals (δ 71.39, 71.71, 74.11, 82.15, 82.48, 82.79, 83.29) in NMR indicated that **16** was an (A) type acetogenin possessing two tetrahydrofuran rings and three hydroxyls.

Table 17. Physico-chemical properties of squamocin-B (**16**)

| | |
|-----------------------------------------|----------------------------------------------------------------------------------------------------------|
| State | White wax |
| M.W. | 594 (FAB-MS: MH ⁻ 595) |
| M.F. | C ₃₅ H ₆₂ O ₇ (HR-FAB-MS: 595.4592 (MH ⁺ , Calcd. 545.4574)) |
| $[\alpha]_D^{25}$ | +27.6 ($c=0.25$, MeOH) |
| UV (λ_{max} , log ϵ) | 209 nm (3.7) |
| IR (ν_{max}) | 3660, 3575, 3450, 1745 (cm ⁻¹ , CHCl ₃) |
| CD | $\Delta\epsilon$ -0.42 (MeOH, at 240 nm) |

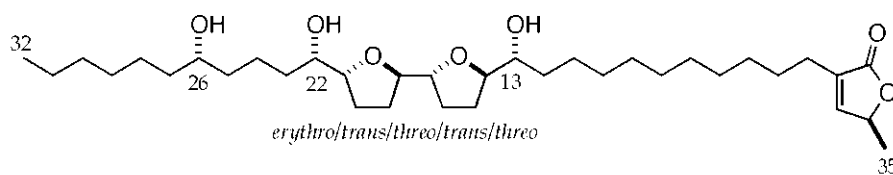
From the EI-MS spectra fragment pattern (Fig. 46), it was clear that a tetrahydrofuran portion was positioned on C-13/C-22, and the other hydroxyl was positioned at C-26. The existence of 1-5 diol relationship between C-22 and C-26 was supported by ¹³C-NMR signal δ 21.99 (C-24).

Fig. 46. EI-MS fragmentation pattern of squamocin-B (**16**)

The ^1H - and ^{13}C -NMR data of this compound (**16**) were very close to that of squamocin (**2**). Further, ^1H -NMR data of its (*R*)-MTPA ester were close to squamocin (*R*)-MTPA ester, too. From above observation, this compound's relative stereochemistry of the tetrahydrofuran portion was determined to be *threo/trans/threo/trans/erythro* relationships. Further, its absolute stereochemistry was determined to be $13R,14R,17R,18R,21R,22S,26S$. The absolute stereochemistry at C-26 of **16** was confirmed as *S* according to the advanced Mosher ester method described above (P. 108).

The absolute chemistry at C-34 was determined to be *S* from CD spectra same as other compounds. Thus, all stereogenic carbinol centers of **16** was determined to be $13R,14R,17R,18R,21R,22S,26S,34S$.

The structure of squamocin-B (**16**) was illustrated as bellow, again a rare C35 acetogenin.



Squamocin-B

(6) Structure of squamostanal-A (**17**)

As shown in Table 18, molecular weight of this compound (**17**) was 294, and the molecular formula was $\text{C}_{18}\text{H}_{30}\text{O}_3$. An existence of α,β -unsaturated- γ -lactone of (I) type was confirmed by UV, ^1H - and ^{13}C -NMR. The oxymethyne signals of NMR, observed in general tetrahydrofuranic acetogenins conventionally, was not observed, but a new aldehyde signal (δ_{H} 9.77 (t, $J=1.9$ Hz, H-15), δ_{C} 203.03 (C-15)) was observed. Since this aldehyde proton signal was coupled with methylene protons of δ_{H} 2.42, and signals δ_{C} 43.90 (C-14) and 22.05 (C-13), it was assumed that the aldehyde was positioned at the terminal of a hydrocarbon chain. The EI-MS spectrum of **17** exhibited fragment ions at m/z 265 (C-14/C-15), 251 (C-13/C-14), 112 (C-3/C-4 +H; McLafferty rearrangement), 111 (C-3/C-4), 97 (C-2/C-3) and 69 (C-2/C-3 -CO) as shown in Fig. 47.

Table 18. Physico-chemical properties of squamostanal-A (**17**)

| | |
|--------------------------------------------|-------------------------------------------------------------------------------------------------|
| State | oil |
| M.W. | 294 (FAB-MS: MH^+ 595) |
| M.F. | $\text{C}_{18}\text{H}_{30}\text{O}_3$ (HR-FAB-MS: 295.2225 (MH^+ , Calcd. 295.2273)) |
| UV (λ_{max} , ϵ) | 210 nm (7000) |
| CD | $\Delta\epsilon$ -0.42 (MeOH, at 240 nm) |

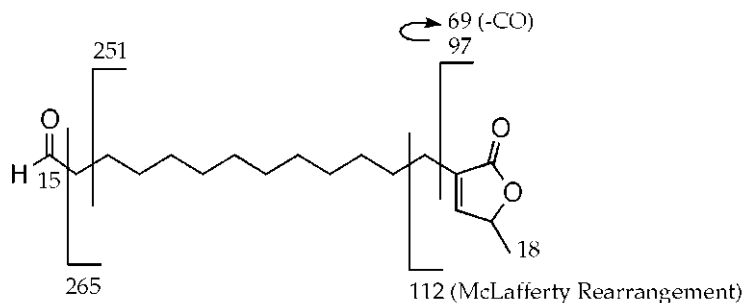
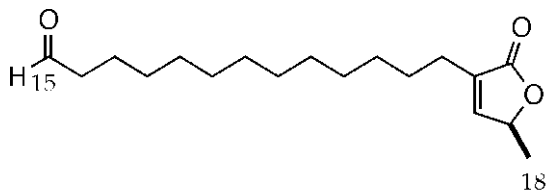


Fig. 47. EI-MS fragmentation pattern of squamostanal-A (**17**)

The asymmetric carbon at C-17 was determined to be *S* configuration because of negative Cotton effect at 239 nm same as in squamocin (**2**). Thus the structure of **17** was elucidated as illustrated, and was named squamostanal-A.

Squamostanal-A (**17**) could be derived from normal C37 acetogenins by an oxidative cleavage between C-15 and C-16. Kawazu et al. obtained a compound with shorter chain by two methylene units than that of **17** by a lead tetraacetate oxidation of neoannonin (= squamocin-J) (Kawazu et al., 1989). ¹H-NMR of the compound was very close to that of **17**.



Squamostanal-A

Chapter Results and Discussion

1. The amine method

The author has established a new method (the amine method was described in Chapter), combining a derivatization with *N,N*-dimethylethylenediamine and the precursor ion scanning method, for planar structure elucidation of annonaceous tetrahydrofuranic acetogenins. Previously, there were still ambiguities of the structure elucidation on the basis of a routine traditional spectral data analysis; such as NMR and MS. On the other hand, this method makes planar structure elucidation of acetogenin easy and reliable.

The precursor ion scanning method has been mainly used for analyses of peptide sequences. This work was the first instance of a successful application of precursor ion scanning to structure elucidation of natural products. A benefit of this method is that planar structure can be elucidated with minute amount of derivative (a few μg). One of the keys to the success of present study was the chain-like structural feature of the tetrahydrofuranic acetogenins. Therefore, this method can be also applied to other natural products such as polycyclic ethers; maitotoxin (Murata, et al., 1993), brevetoxin B (Lin et al., 1981), ciguatoxin (Murata et al., 1990), etc.

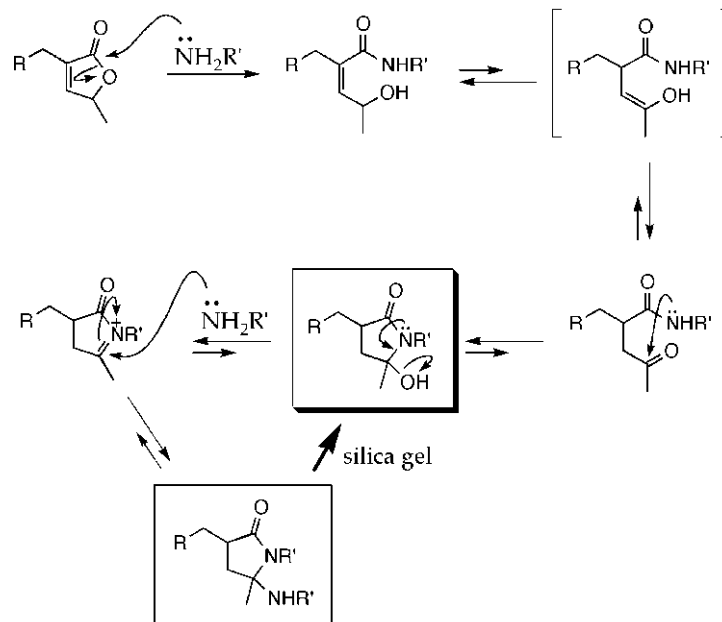
When this method was applied to acetogenins containing double bonds, carbonyl groups, or epoxide, etc., still some issue came out. That is,

- (1) When this method was applied to squamosten-A (**13**), containing a double bond in the hydrocarbon chain, fissions indicating the position of the bond were not observed clearly. However, this is not a major problem since the location of the double bond can be easily determined, from diagnostic ions resulting from single carbon-carbon bond.
- (2) When this method was applied to annonacin-10-one (**10**) (Fig. 34), having a carbonyl group in the hydrocarbon chain, diagnostic fragment ions from fission at beta to carbonyl group were detected intensively. On the hand, fragment ions from fission at alpha to carbonyl group were not detected as an intense peak. The observation of this fragmentation pattern may be specific for carbonyl in acetogenins. Further investigation will be required about other oxo-acetogenins.
- (3) The method has not been applied to a tri-tetrahydrofuran acetogenin or non-classical (E) type acetogenins.
- (4) Derivatization of (b)-(d) type γ -lactone was not investigated.

It is necessary to investigate with known natural compounds together with synthetic compounds for the purpose of solving above problems. Problems (1) and (2) should be solved by measuring at high resolution mode.

Proposed reaction mechanism of derivatization of γ -lactone with amine is shown in Fig. 48.

In recent years, several research groups applied product ion scanning based on charge remote fragmentation to chain like natural compounds (*e.g.*, polyamine, polycyclic ether) (Shinada et al., 2001; Yasumoto et al., 2000). Charge remote fragmentation is a kind of MS/MS pattern that provides simple sequential fragment patterns to make the structure elucidation easy. In general, to observe these charge remote fragmentation patterns, the molecule needs to possess a strong ionic functional group, *e.g.*, sulphonic acid, or ammonium salt, at either molecular terminal. Product ion scanning from molecular ion having a stable charge site at its terminal gives simple sequential fragmentation pattern which enable planar structure to be determined (Fig. 49 (A)). Although the α,β -unsaturated- γ -lactone could be a charge site, typical charge remote fragmentation pattern was not observed in squamocin molecules (Fig. 13). It is thought that the functional group has fail to retain the stable charge, and charge-driven reactions compete with typical charge remote fragmentation. The introduction of amine residue to the molecule is effective for both fixation of charge site at

Fig. 48. Proposed reaction mechanism of γ -lactone portion with amine

the end of molecule and distinction from various fragment ions not containing the residue, which resulted in success of precursor ion scanning of derivatized annonaceous tetrahydrofuranic acetogenins. Furthermore, newly attached amine structure might act as a "tag". That is, the application of precursor ion scanning method make it possible to detect precursor ions containing amine structure which are not charged (Fig. 49 (B)). The concept using a tag in MS/MS, which is a precursor ion scanning from m/z of tag, will be developed and may supplant conventional method.

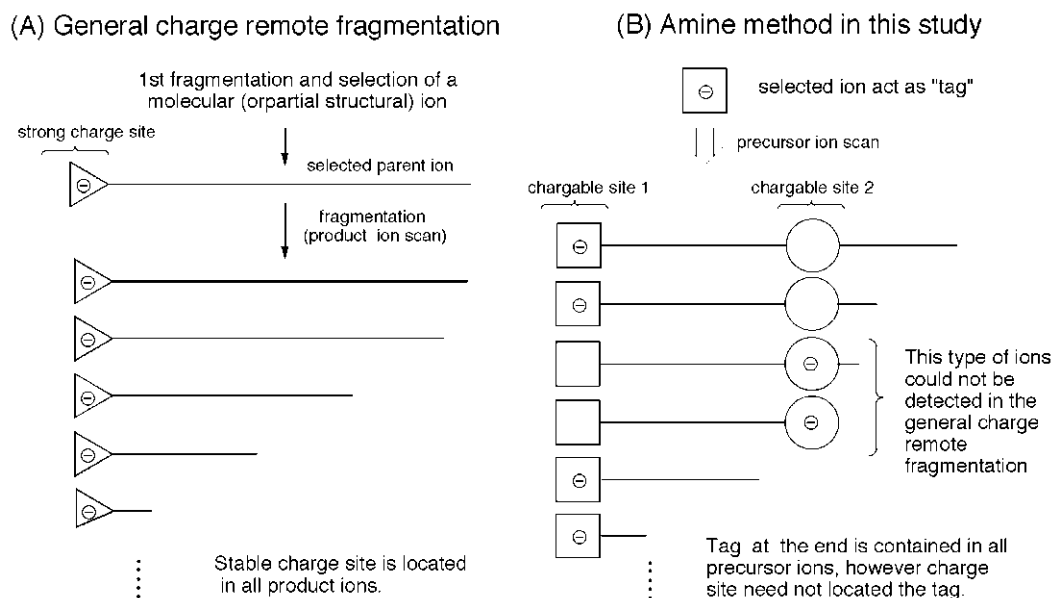


Fig. 49. Difference between product ion scanning based on charge remote fragmentation and precursor ion scanning based on the concept of "tag" (newly developed amine method)

2. New tetrahydrofuranic acetogenins isolated from *Annona squamosa* L. seeds

In this study, seven new tetrahydrofuranic acetogenins, squamocins-B (**16**), -E (**15**), -N (**14**), -O₁ (**11**), -O₂ (**12**),

squamosten-A (**13**) and squamostanal-A (**17**) (Fig. 35), were isolated, and their absolute structures were elucidated as described in Chapter . Sixteen known compounds, squamocins (**2**), -C (**7**), -D, -F (**8**), -G, -H, -I, -J, -K, -L, -M, squamostatins-A (**3**), -B, -C, -D and -E (Fig. 35), were also isolated. The structures of these new acetogenin were elucidated by various spectroscopic methods, including the amine method, advanced Mosher Method (Ohtani, et al., 1991), and derivatization technique etc.

The new type of acetogenins possessing novel structural features among the isolated compounds are: Squamosten-A (**13**); containing a double bond in the hydrocarbon chain, squamostanal-A (**17**); a-chain fragment cleaved at a glycol portion next to tetrahydrofuran, squamocins-O₁ (**11**) and -O₂ (**12**); isomeric pair at C-12 hydroxyl group in the hydrocarbon chains. These structural features play a key role in solving their biosynthetic pathway, especially acetogenin **13**.

3. Biosynthesis of tetrahydrofuranic acetogenins

Since annonaceous tetrahydrofuranic acetogenins belong to fatty acid derivative, these would have been biosynthesized by the type I polyketide synthases (Hopwood et al., 1990). The structure of tetrahydrofuran portion will be formed from precursor having double bonds via epoxide intermediates. This hypothesis is supported by the existence of type (E) non-tetrahydrofuranic acetogenins.

Corepoxylone, isolated from *Annona muricata* L. seeds, was easily converted to corossolone with a treatment of perchloric acid (Fig. 50) (Gromek et al., 1993). Annojahnin, isolated from *A. jahnii* twigs, was converted to epoxide with *m*-CPBA, followed by a perchloric acid treatment to yield 4-deoxy-18/21-(*cis/trans*)-annomontacin-10-one (Fig. 50) (Saszarbitoria et al., 1998).

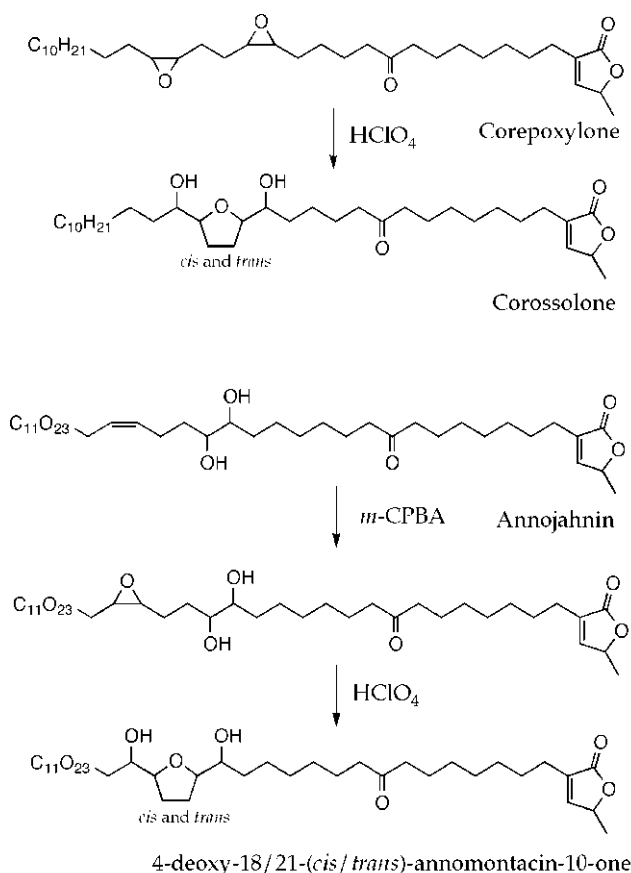


Fig. 50. Semi-synthesis of mono-tetrahydrofuranic acetogenins

Since most of tetrahydrofuranic acetogenins, isolated from *A. squamosa* L. seeds in this study, have 15*R* configuration (13*R* in C35 acetogenins), the biosynthetic pathway of the tetrahydrofuranic portion can be described as in Fig. 51.

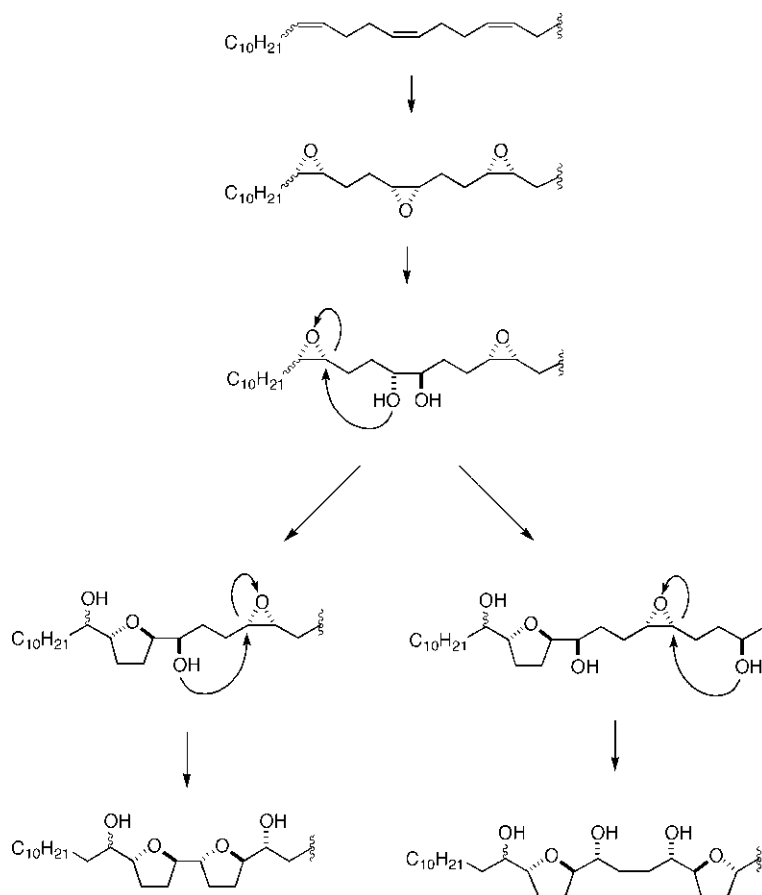


Fig. 51. Proposed biosynthetic pathway of tetrahydrofuran moiety *Annona squamosa* L. seeds

The biosynthesis of α,β -unsaturated- γ -lactone core is an interesting issue. This portion must have formed by addition of C-3 or C-5 units to fatty acid.

Several natural products containing α -substituted- α,β -unsaturated- γ -lactone were reported (Yamada et al., 1995; Schmitz et al., 1966), but biosynthetic study on these compounds was not reported except for acaterin, produced by *Pseudomonas* sp. (Naganuma et al., 1992). Interestingly, its absolute stereochemistry of lactone and hydroxyl group in γ -lactone moiety is opposite to tetrahydrofuranic acetogenin.

In order to elucidate the biosynthetic pathway, induction of callus from leaves of *A. squamosa*, *A. cherimolia* and *A. muricata* were tried (referred to Chapter). As a result, well growing callus was obtained from *A. cherimolia* (Calli of *A. squamosa* and *A. muricata* does grow little). Then, the author examined whether callus of *A. cherimolia* produce acetogenins, however, acetogenin production was not detected.

The biosynthetic pathway of acaterin was investigated by Dr. Y. Sekiyama et al. (Sekiyama et al., 1997; 1998; 1999; 2001). The proposed biosynthetic pathway is outlined in Fig. 52: an initial coupling of acetate (or malonate) and glycerol (or its derivative) leading to a 3,5-dihydroxy-2-penten-4-olide, substitution at C-2 of the lactone with octanoate, dehydration of 5-hydroxy group followed by reduction at C-3 leading to 4-hydroxyacaterin, and final reduction of 4-hydroxyacaterin to acaterin.

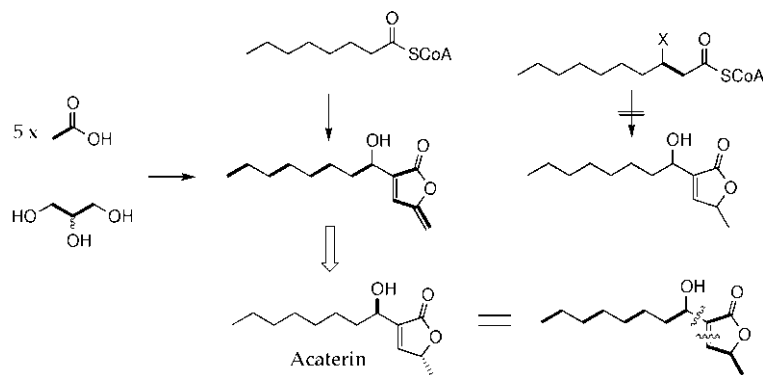


Fig. 52. Biosynthetic pathway of acaterin

4. Bioactivities of tetrahydrofuranic acetogenins

Among the wide spectral bioactivities of tetrahydrofuranic acetogenins pharmaceutical activities are most interesting. Twenty three compounds containing seven new acetogenins were isolated in this study. These have various physico-chemical and structural properties. Structure-activity relationship gave some interesting results. The cytotoxicity against L1210 cell was tested for some acetogenins (Table 19). All compounds tested showed relatively high cytotoxic activity.

Table 19. Cytotoxicity of tetrahydrofuranic acetogenins against L1210

| Compound | Type | ED ₅₀ (µg/ml) |
|----------|---------------------------------|--------------------------|
| Sq-C | (A), C37, <i>th/tr/th/tr/er</i> | 1.0 x 10 ⁻⁵ |
| Sq-G | (A), C37, <i>th/tr/th/tr/er</i> | 1.0 x 10 ⁻⁴ |
| Sq-H | (A), C37, <i>th/tr/th/tr/th</i> | 1.3 x 10 ⁻⁴ |
| Sq-I | (A), C35, <i>er/tr/th/tr/th</i> | 2.1 x 10 ⁻³ |
| Sq-J | (A), C35, <i>th/tr/th/tr/er</i> | 3.3 x 10 ⁻³ |
| Sq-L | (A), C37, <i>th/tr/th/tr/er</i> | 2.0 x 10 ⁻⁴ |
| St-D | (B), C37, <i>tr/th-th/tr/er</i> | 2.2 x 10 ⁻⁴ |
| St-E | (B), C37, <i>tr/th-th/tr/th</i> | 3.8 x 10 ⁻⁴ |

McLaughlin et al. have been investigating bioactivities of many tetrahydrofuran acetogenins, and reported the following general structure-bioactivity relationships (Rupprecht et al., 1990; Fang et al., 1993b; Gu et al., 1995; Zeng et al., 1996; Alali et al., 1999).

- (1) Generally, intensity of the bioactivity was (A) type (adjacent bis-THF) > (B) type (non adjacent bis-THF) > (C) type (mono-THF) > (D) type (non-THF).
- (2) γ -Lactone is crucial for activity.
- (3) If all other structural features are identical, C35 acetogenins are more potent than the C37 acetogenins.
- (4) Thirteen carbons space between the OH-flanked THF and γ -lactone is optimum for activity.
- (5) Three hydroxyl groups, two flanking the THF ring(s) and another somewhere in the long hydrocarbon chain, provide both the optimal position and polarity needed for the most potent activity, and for tetra-hydroxylated acetogenins the activity drops drastically.
- (6) Neither the 4-OH group nor the 10-OH group is essential for activity.
- (7) A ketone instead of a hydroxyl functional group decreases the activity.

- (8) Derivatives (acetates, chloride, etc.) decrease the activity.
 (9) Ketolactone acetogenins are usually less active and more selective than their parent compounds.
 (10) The THP ring compounds are as active as the THF compounds and have the same mechanism of action

Several groups have clarified the mode of action of acetogenins. Londershausen et al. revealed that acetogenins act to respiratory chain of mitochondria, and inhibit site I of electron transport system (Londershausen et al., 1991).

Meanwhile, tetrahydrofuranic acetogenins resemble to monensin well-known as an ionophore, from the viewpoint of consecutive oxygenated functionality containing tetrahydrofurans. An ionophore assay was examined for squamocin (**2**), squamocin-G, squamostatin-A (**3**) (Table 20). Unexpectedly, activity as an ionophore was little, although some researcher reported a chelate effect to metal ions, later (Sasaki et al., 1995; 1998; Hoppe and Scharf, 1995).

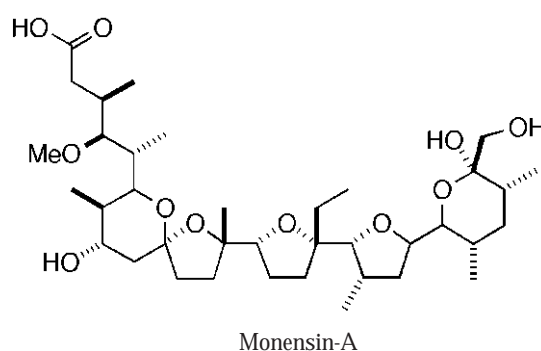


Table 20. Ionophore assay of tetrahydrofuranic acetogenins

| W-08 method compounds | Na ⁺ nmol | Ca ²⁺ nmol |
|---------------------------|-------------------------|--------------------------|
| sq (2) | 0.9x10 ² | 13.9 |
| sq-G | 1.0x10 ² | 13.8 |
| st-A (3) | 0.5x10 ² | 27.7 |
| monencin | 23.1x10 ² | - |
| calcium ionophore (Fluka) | - | 348.6 |
| phosphatidic acid | - | 32.0 |

Since information of biological activity of annonaceous tetrahydrofuranic acetogenin to plant seedlings have not been reported, inhibitory activity of several acetogenins to lettuce (*Lactuca sativa* L.) seedling growth were investigated (Fig. 53) (Aspinall et al., 1967). Interestingly, four adjacent bis-tetrahydrofuranic acetogenins did inhibited lettuce seedling growth, and squamostatin-A (**3**), non-adjacent bis-tetrahydrofuranic acetogenin only exhibited inhibitory activity.

Recently, McLaughlin's group demonstrated that acetogenins act as a chemical defense in the zebra swallowtail butterfly, *Eurytides marcellus* (Martin et al., 1999). *Asimina triloba* produce various annonaceous tetrahydrofuranic acetogenins, showing potent pesticidal and antineoplastic activity. The larvae of the butterfly have resistance against toxicity of acetogenins. They revealed that larvae and mature butterfly had four annonaceous acetogenins to resist against bird predation.

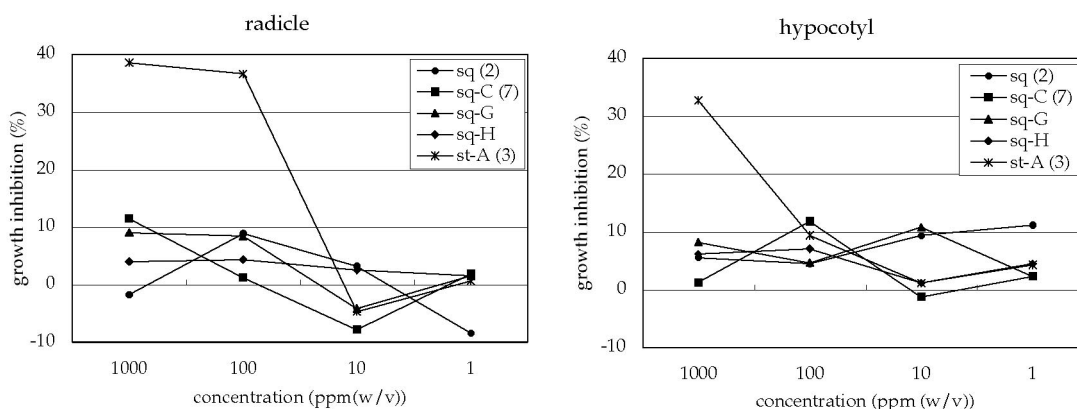


Fig. 53. Inhibitory activity of annonaceous tetrahydrofuranic acetogenins to lettuce seedling growth

5. Remained problems and a prospect in the future

Studies about tetrahydrofuranic acetogenins have been made rapid progress in the last ten years, and these have been attracted multidisciplinary research. Some problems however still remained.

- (1) Absolute configuration of all the compounds has not been solved completely (Rupprecht et al., 1990; Fang et al., 1993b; Gu et al., 1995; Zeng et al., 1996; Alali et al., 1999).
- (2) Total syntheses have been archived for only several acetogenins (Hoppe et al., 1995; Figadere, 1995; Peyrat et al., 1997; Casiraghi et al., 1998).
- (3) Although the annonaceous acetogenins are promising new antitumor and pesticidal agents, all of acetogenins were not tested systematically for their activity.

It is hoped that these problems will be solved in the near future, but the reason for existence of tetrahydrofuranic acetogenins in limited species will remain unexplained yet.

Chapter Experimental Section

General experimental procedures

Melting points were determined on a YAZAWA BY-1 hot-stage microscope and are uncorrected. The Nuclear Magnetic Resonance (NMR) spectra were recorded on a JEOL GSX-500 (^1H at 500 MHz and ^{13}C at 125 MHz), JEOL EX-400 (^1H at 400 MHz and ^{13}C at 100 MHz), JEOL GSX-270 (^1H at 270 MHz and ^{13}C at 67.5 MHz) or Varian INOVA-400 (^1H at 400 MHz and ^{13}C at 100 MHz) spectrometer with CDCl_3 as solvent and tetramethylsilane (TMS) as an internal reference. UV spectra were obtained on a Shimadzu UV-200 spectrometer in MeOH solution. CD spectra were measured with a JASCO J-500C polarimeter at 25 °C in MeOH solution. IR spectra were determined on a JASCO IR-810 spectrometer in CHCl_3 solution. Optical Rotations were measured with a JASCO DIP-360 polarimeter at 25 °C in MeOH solution. EI- (70 eV) and FAB-MS were obtained with a JEOL JMS-AX505HA spectrometer. *m*-Nitrobenzyl alcohol was used as the matrix for the measurement of FAB-MS. Precursor-ion spectrum (FAB-MS/MS) was measured with a Finnigan-MAT TSQ-700 mass spectrometer. Elemental analysis was done on a Perkin-Elmer 240 analyzer. Column chromatography was carried out on Kiesel gel 60 (70-230 mesh, Merck). Thin layer chromatography was carried out on Kiesel gel 60 F₂₅₄ (0.25 mm or 0.5 mm, Merck). HPLC was performed on a Shimadzu LC-6A apparatus equipped with an SPD-6A UV detector (220 nm). A Shimadzu Shim-pack CLC-ODS column (150 mm × 6 mm i.d.) and an STR Prep-ODS column (250 mm × 20 mm i.d.) were used for analytical and preparative purposes, respectively.

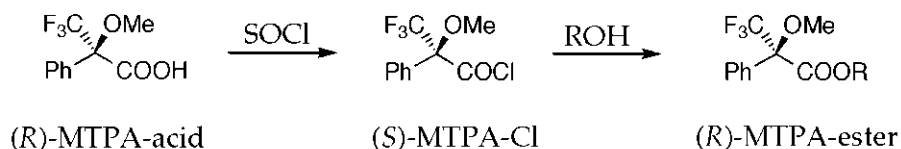
Preparation of acetate derivative (Greene and Wuts, 1999)

Acetogenin (*ca.* 2 mg) was treated with acetic anhydride (20 μl) and anhydrous pyridine (20 μl) in a micro tube at room temperature for overnight. MeOH (20 mg) was added to the mixture and the solvent was removed by flushing with nitrogen gas stream. Separation of the product by a silica gel p-TLC (generally used Hexane : AcOEt = 2 : 1 as development solvents) afforded a purified acetate as an oil.

Preparation of (*R*)- and (*S*)-MTPA ester (Ohtani et al., 1991)

The (*R*)-MTPA ester was prepared according to a slight modification of the Ohtani's method. Pyridine (20 μl) and (*S*)-(+)-MTPA chrolide (8 μl) were added to *ca.* 2 mg of acetogenin. Precipitation of the hydrochloride salt occurred immediately. After *ca.* an hour (completion of the reaction was confirmed by TLC), [3-(dimethyl)amino]propylamine (5 μl) was added. After a few minutes, the mixture was diluted with AcOEt. Separation of the mixture by p-TLC (generally used Hexane : AcOEt = 2 : 1 as a development solvent) furnished the purified (*R*)-MTPA ester. The (*S*)-MTPA ester was similarly prepared using (*R*)-(-)-MTPA chloride.

Cahn-Ingold-Prelog priority (Cahn et al., 1966) interchanges in chemical conversions of the MTPA acid to MTPA-Cl and MTPA-Cl to the MTPA ester.



Synthesis of derivative (4)

Squamocin (**2**) 50 mg, benzylamine 50 μg were sealed in a microtube, and stand at 90 °C twenty hours. After

addition of a small amount of AcOEt, excess benzylamine was removed in vacuo with heating. The residue was chromatographed on silica gel (CHCl₃/AcOEt/MeOH= 10 : 4 : 1, 2 : 3 : 1, 0 : 1 : 1), followed by HPLC (MeOH, flow rate: 6.0 ml/min, UV: 220 nm, retention time: 13.7 min, squamocin: 11 min) purification afforded derivative **4** (30 mg). State: Waxy solid, HR-FAB-MS: Found, 819.6248 (Calcd for C₅₁H₈₃O₆N₂, 819.6251), IR ν_{\max} (CHCl₃) cm⁻¹: 3580, 3440, 3010, 2930, 2955, 1665, 1410, 1240, 1060, 705 cm⁻¹, ¹H-NMR (500 MHz, CDCl₃), ¹³C-NMR (125 MHz, CDCl₃): Table 4.

Synthesis of derivative (5)

(i) Derivatization under neat condition

(a) Squamocin (**2**) 10 mg was dissolved to *N,N*-dimethylethylenediamine (10 μ l) in a micro tube, heating on an oil bath at 80 °C for four hours. After dilution with a small amount of ethyl acetate, the mixture was purified with silica gel p-TLC (develop solvent; CHCl₃ : MeOH= 6 : 1, twice). The bands of R_f value 0.66, 0.52 were scraped off, and eluted with CHCl₃ : MeOH = 1 : 1, which afforded compounds **5a** and **5b** respectively. Both of these compounds were mixture of two compounds (yield; 2 mg, cited in Chapter). R_f values of **5a**, **5b** on the silica gel TLC were 0.24, 0.16 (AcOEt/CHCl₃/MeOH= 2 : 3 : 1) respectively. MW: 710, MF: C₄₁H₇₈O₇N₂, State: wax, HR-FAB-MS: Found, 711.5941 (Calcd for C₄₁H₇₉O₇N₂, 711.5887), IR ν_{\max} (CHCl₃) cm⁻¹: 3430, 3010, 2930, 2860, 1670, 1460, 1400, 1065 cm⁻¹, ¹H-NMR (500 MHz, CDCl₃), ¹³C-NMR (125 MHz, CDCl₃): Table 5.

(b) Squamocin (**2**) 50 mg was dissolved to *N,N*-dimethylethylenediamine (50 μ l) in a micro tube, heating on a 80 °C oil bath for four hours. The mixture was purified on the silica gel column chromatography (3g, CHCl₃ : MeOH= 1 : 1) and afforded compound **5** (11 mg, yield: 22 %).

(c) After reaction was performed, the mixture was added small amount of ethyl acetate. Then, the excess reagent was removed with warming by a vacuum pump. The residue was purified on the silica gel column chromatography, and afforded **5** (11 mg, 22 %) from CHCl₃ : MeOH=1 : 1 fractions. Further, as **5a**, **5b** were noticed in eluted fractions of CHCl₃ : MeOH=2 : 1, this fraction was repurified with silica gel column chromatography, then 28 mg of **5** was obtained, and combined with above **5** (total yield: 56 %).

(ii) Derivatization in dioxane

Squamocin (**2**) 10 mg, *N,N*-dimethylethylenediamine 0.5 μ l were dissolved in dioxane 0.5 ml, and heated in an oil bath at 80 °C for eight hours. The derivative (**5**) (ca. 50 %) and starting material **2** (ca. 50 %) were observed on the TLC of the mixture, but the derivative (**6**) was not observed at all.

Synthesis of derivative (6)

Squamocin (**2**) 20 mg was dissolved in *N,N*-dimethylethylenediamine 20 μ g in a micro tube, and heated at 80 °C for four hours. The reactant was dissolved in a small amount of ethyl acetate, followed by transference to a nasu-flask, and an excess reagent was removed in vacuo with heating. This crude mixture was not purified, because reactant was adsorbed on silica gel or ODS strongly (these chromatography elution were tried with methanol as an eluant). A series of spectral experiments were performed to the reactant mixture. Molecular weight: 780, Molecular formula: C₄₅H₈₈O₆N₄, State: waxy solid, HR-FAB-MS: Found, 781.6730 (Calcd for C₄₁H₇₉O₇N₂, 781.6782), IR ν_{\max} (CHCl₃) cm⁻¹: 3430, 3010, 2930, 2860, 2780, 1670, 1465, 1415, 1380, 1055, 910 cm⁻¹, ¹H-NMR (500 MHz, CDCl₃), ¹³C-NMR (125 MHz, CDCl₃): Table 6.

Squamocin (2)

Squamocin (**2**) was obtained as a major acetogenin from *A. squamosa* L. seeds. A series of reactions performed

to this compound has physico-chemical property as given bellow.

State: white needles, MP: 48.5-49 °C, $[\alpha]_D^{25}$: +15.0 (c=1.7, MeOH), UV λ_{max} (MeOH) nm (log ϵ): 215 (3.5), IR ν_{max} (CHCl₃) cm⁻¹: 3680, 3585, 3460, 3015, 2940, 2855, 1755, ¹H-NMR (500 MHz, CDCl₃) δ : 0.83 (3H, t, J =7.0 Hz, H-34), 1.36 (3H, d, J =6.8 Hz, H-37), 2.21 (2H, tt, J =7.7, 1.4 Hz, H-3), 3.33 (1H, m, H-15), 3.52 (1H, m, H-28), 3.76 (3H, m, H-16, -23, 24), 3.86 (2H, m, H-19, -20), 4.95 (1H, qq, J =6.8, 1.4 Hz, H-36), 6.96 (1H, d, J =1.4 Hz, H-35), ¹³C-NMR (125 MHz, CDCl₃) δ : 14.1 (C-34), 19.2 (C-37), 22.0 (C-26), 22.6 (C-33), 24.8 (C-22), 25.2 (C-3), 25.7 (C-13, -30), 27.4 (C-4), 28.4 (C-17), 28.9 (C-18, -21), 29-30 (C-6, -7, -8, -9, -10, -11, -12), 29.2 (C-5), 29.7 (C-31), 31.8 (C-32), 32.5 (C-25), 33.3 (C-14), 37.3 (C-27), 37.5 (C-29), 71.4 (C-24), 71.8 (C-28), 74.1 (C-15), 77.4 (C-36), 82.2 (C-19, or -20), 82.5 (C-20 or -19), 82.8 (C-23), 83.3 (C-16), 134.3 (C-2), 148.8 (C-35), 173.9 (C-1).

(*R*)-MTPA ester of squamocin (2r)

¹H-NMR (500 MHz, CDCl₃) δ : 0.874 (3H, t, J =7.1 Hz, H-34), 1.41 (3H, d, J =6.4 Hz, H-37), 2.264 (2H, t, J =6.8 Hz, H-3), 3.513, 3.531, 3.609 (3H each, s, OMe), 3.65 (1H, m, H-19), 3.83 (1H, m, H-20), 3.87 (1H, m, H-23), 3.99 (1H, m, H-16), 4.99 (1H, q, J =6.6 Hz, H-36), 5.02 (2H, m, H-15, -28), 5.14 (1H, m, H-24), 6.98 (1H, br s, H-35), 7.33-7.65 (15H, m, aromatic).

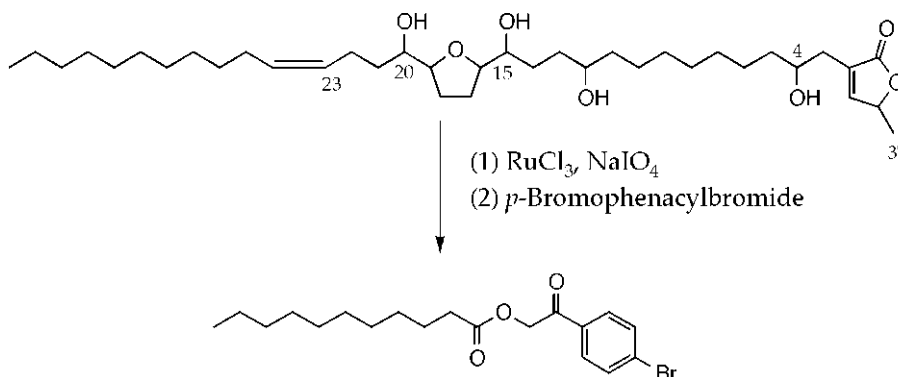
(*S*)-MTPA ester of squamocin (2s)

¹H-NMR (500 MHz, CDCl₃) δ : 0.860 (3H, t, J =7.1 Hz, H-34), 1.40 (3H, d, J =6.4 Hz, H-37), 2.263 (2H, t, J =6.9 Hz, H-3), 3.510, 3.534, 3.546 (3H each, s, OMe), 3.79 (2H, m, H-19, -20), 3.96 (1H, m, H-23), 4.03 (1H, q, J =6.7 Hz, H-16), 4.99 (2H, m, H-28, -36), 5.06 (1H, m, H-15), 5.20 (1H, q, J =6.6 Hz, H-24), 6.98 (1H, br s, H-35), 7.33-7.64 (15H, m, aromatic).

Oxidation of squamosten-A (13) with ruthenium () chloride and following *p*-bromophenacyl esterification of the fatty acid

Squamosten-A (12) (55 μ g), sodium periodate (100 μ g), ruthenium () chloride-n-H₂O (catalytic amount) were dissolved in H₂O-CH₃CN-CCl₄ solvent mixture (10 μ l each) and stirred an hour (Carlsen et al., 1981). The reaction mixture was partitioned between aqueous NaHCO₃ and CCl₄, and after acidification of aqueous layer by use of 2N-HCl, extracted with ether. The ether layer concentration afforded mixture of fatty acids.

p-Bromophenacyl bromide (200 μ g), acetonitrile (100 μ l), *N,N*-diisopropylethylamine (5 μ g) were added to the mixture and stand at room temperature two hours (Tokyo Kasei Kogyo), and afforded fatty acids *p*-bromophenacyl esters. The analytical HPLC condition: column; TSK-gel (ODS-120T, 250 mm \times 3 mm i.d.), eluent: CH₃CN/H₂O (20 : 1), flow rate; 0.5 ml/min, UV; 254 nm. [Retention times of authentic samples in this condition: phenacyl octate 9.1 min; phenacyl nonate 10.1 min; phenacyl decate 11.4 min; phenacyl undecate 13.3 min; phenacyl dodecate 15.7 min (Fig. 42).]



Isolation of acetogenins

Petrol ether extract of seeds of *Annona squamosa* L. was obtained from Baranas Hihdu University, India (Fig. 36).

Squamocin-O₁ (11)

Fraction 10 shown in Fig. 36 was chromatographed with HPLC (column: Shimadzu Shim-Pack ODS (250 cm × 10 mm i.d.); solvent MeOH : H₂O = 10 : 1; flow rate: 1.0 ml/min) and collected a peak of retention time: 12.5 min, and the fraction was rechromatographed with HPLC (MeOH : CH₃CN : H₂O : isopropanol = 120 : 40 : 30 : 1, flow rate: 6.0 ml/min, retention time 39.0 min fraction was collected) (20 mg). MW: 638, MF: C₃₇H₆₆O₆, state: white wax, HR-FAB-MS: *m/z* 639.4792 (Calcd for C₃₇H₆₇O₈ 639.4835), State: white wax (20 mg), $[\alpha]_D^{25}$: +17.7 °(c=0.6, MeOH), UV λ_{max} (MeOH) nm (log ϵ): 210 (3.8), CD (MeOH) $\Delta\epsilon$ (nm) -0.45 (239). IR ν_{max} (CHCl₃) cm⁻¹: 3690, 3585, 3460, 1750. EI-MS *m/z* 620, 602 517, 505, 415, 397, 379, 363, 345, 293, 275, 97. HR-FAB-MS *m/z* 639.4792 [(M+H)⁺] (Calcd for C₃₇H₆₇O₈, 639.4835). ¹H-NMR (500 MHz, CDCl₃) δ : 0.88 (3H, t, *J*=6.4 Hz, H-34), 1.41 (3H, d, *J*=6.4 Hz, H-37), 2.26 (2H, t, *J*=7.8 Hz, H-3), 3.45 (1H, br t, *J*=7.8 Hz, H-15), 3.60 (2H, m, H-12, -28), 3.76-3.96 (5H, m, H-16, -19, -20, -23, -24), 5.00 (1H, qq, *J*=6.8, 1.9 Hz, H-36), 6.99 (1H, s, H-35). ¹³C-NMR (125 MHz, CDCl₃) : Table 7.

Tetra-(*R*)-MTPA ester of squamocin-O₁ (11r)

¹H-NMR (500 MHz, CDCl₃): Table 8.

Tetra-(*S*)-MTPA ester of squamocin-O₁ (11s)

¹H-NMR (500 MHz, CDCl₃): Table 8.

Squamocin-O₂ (12)

Fraction 10 shown in Fig. 36 was chromatographed with HPLC (column: Shimadzu Shim-Pack ODS (250 cm × 10 mm i.d.); solvent MeOH : H₂O = 10 : 1; flow rate: 1.0 ml/min) and collected a peak of retention time: 12.5 min, and the fraction was rechromatographed with HPLC (MeOH : CH₃CN : H₂O : isopropanol = 120 : 40 : 30 : 1, flow rate: 6.0 ml/min, retention time 43.0 min fraction was collected) (9 mg). MW: 638, MF: C₃₇H₆₆O₆, state: white wax, HR-FAB-MS: *m/z* 639.4781 (Calcd for C₃₇H₆₇O₈ 639.4835), State: white wax (9 mg), $[\alpha]_D^{25}$: +17.4 °(c=1.0, MeOH), UV λ_{max} (MeOH) nm (log ϵ): 210 (3.8), CD (MeOH) $\Delta\epsilon$ (nm) -0.45 (239). IR ν_{max} (CHCl₃) cm⁻¹: 3690, 3585, 3460, 1750. The EI-MS spectrum was identical to that of **11**. HR-FAB-MS *m/z* 639.4781 [(M+H)⁺] (Calcd for C₃₇H₆₇O₈, 639.4835). ¹H-NMR (500 MHz, CDCl₃) δ : 0.88 (3H, t, *J*=6.9 Hz, H-34), 1.41 (3H, d, *J*=6.4 Hz, H-37), 2.26 (2H, t, *J*=7.8 Hz, H-3), 3.45 (1H, br t, *J*=7.8 Hz, H-15), 3.58 (2H, m, H-12, -28), 3.76-3.96 (5H, m, H-16, -19, -20, -23, -24), 5.00 (1H, qq, *J*=7.0, 1.8 Hz, H-36), 6.99 (1H, s, H-35). ¹³C-NMR (125 MHz, CDCl₃): Table 7.

Tetra-(*R*)-MTPA ester of squamocin-O₂ (12r)

¹H-NMR: Table 8.

Tetra-(*S*)-MTPA ester of squamocin-O₂ (12s)

¹H-NMR: Table 8.

Squamosten-A (13)

Fraction 7 shown in Fig. 36 was chromatographed by HPLC (MeOH : H₂O = 20 : 1, flow rate: 6.0 ml/min, UV: 220 nm) and collected a peak of retention time: 18.5 min, further the fraction was rechromatographed with HPLC (column: Shiseido Capcell Pak C18 (25 cm × 3 mm, CH₃CN : H₂O = 20 : 1, flow rate: 0.7 ml/min, retention time 9.3 min, UV: 220 nm) and afforded squamosten-A (**13**) as a white crystal (9 mg). MW: 622, MF: C₃₇H₆₆O₇, State: white micro crystals, MP: 64-67, $[\alpha]_D^{25}$: +9.0 (c=0.10, MeOH), CD $\Delta\epsilon$ (nm): -0.57 (240), HR-FAB-MS: Found, 623.4844 (Calcd for C₃₇H₆₇O₇N₂, 623.4887), ¹H-NMR (500 MHz, CDCl₃) δ : 0.88 (3H, t, *J*=7.3 Hz, H-34), 1.2-1.4 (m, methylene), 1.39 (3H, d, *J*=7.3 Hz, H-37), 1.43 (2H, m, H-14, -21), 1.7-1.4 (6H, m, H-5, -11, -13), 1.9-1.7 (4H, dt, *J*=8.8, 7.2 Hz, H-17, -18), 2.04 (2H, q, *J*=7.6 Hz, H-25), 2.17 (2H, m, H-22), 2.40 (1H, dt, *J*=14.6, 8.3 Hz, H-3a), 2.53 (1H, dd, *J*=14.6, 1.8 Hz, H-3b), 3.44 (2H, m, H-15, -20), 3.63 (1H, m, H-12), 3.81 (1H, m, H-4), 3.83 (2H, m, H-16, -19), 5.05 (1H, qd, *J*=7.3, 1.8 Hz, H-36), 5.36 (1H, m, H-23), 5.39 (1H, m, H-24), 7.18 (q, *J*=1.8 Hz, H-35), ¹³C-NMR (125 MHz, CDCl₃) δ : 14.1 (C-34), 19.1 (C-37), 22.7 (C-33), 23.3 (C-22), 25.5 (C-6), 25.6 (C-10), 27.3 (C-8, -25), 28.7 (C-17, -18), 29-30 (C-7, -8, -9, -14, -26, -27, -28, -29, -30, -31), 31.9 (C-32), 33.4 (C-3), 33.6 (C-13, -21), 37.4 (C-5), 37.5 (C-11), 70.0 (C-4), 71.7 (C-12), 73.5 (C-20), 74.4 (C-15), 78.0 (C-36), 82.6 (C-16), 83.7 (C-19), 128.9 (C-23), 130.9 (C-24), 131.2 (C-2), 151.8 (C-35), 174.6 (C-1).

Tetra-(*R*)-MTPA ester of squamosten-A

¹H-NMR (500 MHz, CDCl₃) δ : 0.88 (3H, t, *J*=7.0 Hz, H-34), 1.31 (3H, d, *J*=7.3 Hz, H-37), 1.89 (2H, m), 2.60 (1H, br d, *J*=13.0 Hz, H-3a), 2.68 (1H, *J*=15.4, 7.7 Hz, H-3b), 3.48, 3.50, 3.51, 3.53 (3H each, s, OMe), 3.93 (1H, q, *J*=6.8 Hz, H-19 or H-16), 4.02 (1H, m, H-16 or H-19), 4.91 (1H, m, H-36), 4.98, 5.04 (1H each, m, H-15, -20), 5.04 (1H, m), 5.22 (1H, q-like, *J*=10.6 Hz, H-23), 5.37 (2H, m, H-4, -24), 6.96 (1H, s, H-35), 7.31-7.63 (20H, m, aromatic).

Squamocin-N (**14**)

Fraction 1 shown in Fig. 36 was dissolved in a small amount of MeOH, and loaded Sep-Pack C18 cartridge (Waters). Then the solution was chromatographed on ODS HPLC column (MeOH, flow rate: 6.0 ml/min) and collected a retention time=16.9 min peak, and afforded Squamocin-N (**14**) (19 mg). MW: 606, MF: C₃₇H₆₆O₆, State: white wax, $[\alpha]_D^{25}$: +40.6 (c=0.43, MeOH), CD (MeOH) $\Delta\epsilon$ (nm): -0.57 (240), elemental analysis: Anal. C, 73.01; H, 11.25 (Calcd for C₃₇H₆₆O₆ C, 73.22; H, 10.96), ¹H-NMR (500 MHz, CDCl₃) δ : 0.88 (3H, t, *J*=7.1 Hz, H-34), 1.41 (3H, d, *J*=6.7 Hz, H-37), 2.26 (2H, t, *J*=7.7 Hz, H-3), 3.40 (2H, qui, *J*=4.3 Hz, H-15, H-24), 3.81-3.85 (2H, q, *J*=6.0 Hz, H-16, -23), 3.88-3.93 (2H, m, H-19, -20), 4.99 (1H, q, *J*=6.8 Hz, H-36), 6.98 (1H, d, *J*=1.3 Hz, H-35), ¹³C-NMR (125 MHz, CDCl₃) δ : 14.1 (C-34), 19.2 (C-37), 22.7 (C-33), 25.2 (C-3), 25.4 (C-26), 25.8 (C-13), 27.3 (C-18, -21), 27.4 (C-4), 29.0 (C-17, -22), 29-30 (C-5, -6, -7, -8, -9, -10, -11, -12, -27, -28, -29, -30, -31), 31.9 (C-32), 34.4 (C-14, -25), 74.0 (C-15, -23, -24), 77.4 (C-36), 81.0 (C-19, -20), 82.8 (C-16), 134.4 (C-2), 148.8 (C-35), 173.9 (C-1).

Squamocin-N acetate

¹H-NMR (500 MHz, CDCl₃) δ : 0.88 (3H, t, *J*=7.1 Hz, H-34), 1.41 (3H, d, *J*=7.0 Hz, H-37), 2.070 (3H, s, AcO), 2.26 (2H, t, *J*=7.7 Hz, H-3), 3.87 (2H, m, H-19, -20), 3.94 (2H, m, H-16, -23), 4.95 (2H, m, H-15, -24), 4.99 (1H, q, *J*=6.9 Hz, H-36), 6.98 (1H, s, H-35).

Di-(*R*)-MTPA ester of squamocin-N

¹H-NMR (500 MHz, CDCl₃) δ : 0.88 (3H, t, *J*=6.8 Hz, H-34), 1.40 (3H, d, *J*=6.8 Hz, H-37), 2.26 (2H, t, *J*=8.1 Hz, H-3), 3.648, (6H, s, OMe), 3.90 (4H, m, *J*=6.4 Hz, H-16, -19, -20, -23), 4.98 (1H, q, *J*=7.0 Hz, H-36), 5.49 (2H, td, *J*=8.5, 3.5 Hz, H-15, -24), 6.98 (1H, d, *J*=1.5 Hz, H-35), 7.34-7.73 (10H, m, aromatic).

Di-(S)-MTPA ester of squamocin-N

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.8$ Hz, H-34), 1.40 (3H, d, $J=6.8$ Hz, H-37), 2.26 (2H, t, $J=7.6$ Hz, H-3), 3.560 (6H, s, OMe), 3.76 (2H, qui, $J=5.0$ Hz, H-19, -20), 3.95 (2H, q, $J=6.5$ Hz, H-16, -23), 4.99 (1H, q, $J=6.6$ Hz, H-36), 5.16 (2H, q, $J=6.3$ Hz, H-15, -24), 6.98 (1H, br s, H-35), 7.35-7.65 (10H, m, aromatic).

Squamocin-E (15)

Sixth fraction shown in Fig. 36 was chromatographed with HPLC (MeOH : $\text{H}_2\text{O}= 20 : 1$, flow rate: 6.0 ml/min) and collected a peak of retention time: 16.7 min, and the fraction was rechromatographed with HPLC ($\text{CH}_3\text{CN} : \text{H}_2\text{O}= 20 : 1$, flow rate: 7.0 ml/min, retention time 21.0 min fraction was collected). Further purification by HPLC (MeOH : $\text{H}_2\text{O} = 13 : 1$, flow rate: 7.0 ml/min, retention time: 19.0 min) afforded squamocin-E (**15**) as a white crystal (54 mg). MW: 594, MF: $\text{C}_{35}\text{H}_{62}\text{O}_7$, State: white solid, MP: 77-78 °C, $[\alpha]_{\text{D}}^{25}$: +20.9 ($c=0.25$, MeOH), CD (MeOH) $\Delta\epsilon(\text{nm})$: -0.40 (240), HR-FAB-MS: 595.4592 (Calcd for $\text{C}_{35}\text{H}_{63}\text{O}_7$ 595.4574), $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.4$ Hz, H-32), 1.43 (3H, d, $J=6.4$ Hz, H-35), 2.40 (1H, dd, $J=15.0, 8.2$ Hz, H-3a), 2.53 (1H, br d, $J=15.0$ Hz, H-3b), 3.39 (2H, m, H-13, -22), 3.82-3.90 (5H, m, H-4, -14, -17, -18, -21), 5.06 (1H, qq, $J=6.9, 1.4$ Hz, H-34), 7.19 (1H, br s, H-33), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.1 (C-32), 19.1 (C-35), 22.6 (C-31), 25.5 (C-6, -11), 25.6 (C-24), 28.3 (C-15, -20), 28.9 (C-16, -19), 29-30 (C-7, -8, -9, -10, -25, -26, -27, -28, -29), 31.9 (C-30), 33.3 (C-3), 33.4 (C-12, -23), 37.3 (C-5), 69.9 (C-4), 74.0 (C-13, -22), 77.9 (C-34), 81.7 (C-17, -18), 83.1 (C-14, -21), 131.1 (C-2), 151.8 (C-33), 174.6 (C-1).

Squamocin-E acetate

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=5.6$ Hz, H-32), 1.39 (3H, d, $J=6.9$ Hz, H-35), 2.54 (2H, m, H-3), 2.025 (3H, s, AcO), 2.076 (6H, s, 2 \times AcO), 3.91 (2H, m, H-17, -18), 3.99 (2H, q, $J=6.3$ Hz, H-14, -21), 4.86 (1H, q, $J=6.0$ Hz, H-34), 5.01 (1H, qq, $J=6.8, 1.7$ Hz, H-34), 5.10 (1H, m, H-4), 7.08 (1H, br s, H-33).

Tris-(R)-MTPA ester of squamocin-E

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.0$ Hz, H-32), 1.31 (3H, d, $J=7.1$ Hz, H-35), 2.60 (1H, br d, $J=15.6$ Hz, H-3a), 2.67 (1H, dd, $J=15.6, 7.8$ Hz, H-3b), 3.499, 3.598, 3.605 (3H each, s, OMe), 3.93 (2H, t, $J=6.5$ Hz, H-17, -18), 4.00 (2H, q, $J=7.0$ Hz, H-14, -21), 4.90 (1H, m, H-34), 5.03 (2H, m, H-13, -22), 5.38 (1H, m, H-4), 6.97 (1H, br s, H-33), 7.33-7.64 (15H, m, aromatic).

Squamocin-B (16)

Ninth fraction shown Fig. 36 was chromatographed with HPLC (MeOH : $\text{H}_2\text{O}= 20 : 1$, flow rate: 6.0 ml/min), and collected a retention time 12.3 min fraction. Further, the fraction was rechromatographed with HPLC ($\text{CH}_3\text{CN} : \text{H}_2\text{O}= 20 : 1$, flow rate: 6.0 ml/min) and collected 12.2 min fraction, followed pTLC ($\text{CHCl}_3 : \text{MeOH} : \text{H}_2\text{O}= 10 : 1$, Rf: 0.6) afforded squamocin-B (**16**) (37 mg), MW: 594, MF: $\text{C}_{35}\text{H}_{62}\text{O}_7$, State: white wax, $[\alpha]_{\text{D}}^{25}$: +27.6 ($c=0.25$, MeOH), CD (MeOH) $\Delta\epsilon(\text{nm})$: -0.42 (240), HR-FAB-MS: 595.4630 (Calcd for $\text{C}_{35}\text{H}_{63}\text{O}_7$ 595.4574), $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.6$ Hz, H-32), 1.41 (3H, d, $J=6.3$ Hz, H-35), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.38 (1H, m, H-13), 3.60 (1H, m, H-26), 3.78-3.89 (3H, m, H-14, -21, -22), 3.89-3.97 (2H, m, H-17, -18), 4.99 (1H, q, $J=7.1$ Hz, H-34), 6.99 (1H, s, H-33), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.0 (C-32), 19.2 (C-35), 22.0 (C-24), 22.6 (C-31), 24.8 (C-20), 25.1 (C-3), 25.6 (C-11, -28), 27.4 (C-4), 28.4 (C-15), 28.9 (C-16, -19), 29-30 (C-6, -7, -8, -9, -10), 29.1 (C-5), 29.7 (C-29), 31.8 (C-30), 32.4 (C-23), 33.2 (C-12), 37.2 (C-25), 37.4 (C-27), 71.4 (C-22), 71.7 (C-26), 74.1 (C-13), 77.4 (C-34), 82.2 (C-17 or -18), 82.5 (C-18, or -17), 82.8 (C-21), 83.3 (C-14), 134.3 (C-2), 148.8 (C-33), 173.9 (C-1).

Tris-(*R*)-MTPA ester of squamocin-B

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.87 (3H, t, $J=7.2$ Hz, H-32), 1.31 (3H, d, $J=6.4$ Hz, H-35), 2.27 (2H, t, $J=6.8$ Hz, H-3), 3.51, 3.53, 3.61 (3H each, s, OMe), 3.65 (1H, m, H-17 or H-18), 3.83 (1H, m, H-18 or H-17), 3.87 (1H, m, H-21), 3.99 (1H, q, $J=7.3$ Hz, H-14), 4.99 (1H, q, $J=6.6$ Hz, H-34), 5.02 (2H, m, H-13, -26), 5.14 (1H, m, H-22), 6.98 (1H, s, H-33), 7.33-7.65 (15H, m, aromatic).

Squamstanal-A (17)

Fifth fraction shown in Fig. 36 was chromatographed on silica gel ($\text{CH}_2\text{Cl}_2/\text{AcOEt}$ solvent system), and fraction eluted with $\text{CH}_2\text{Cl}_2 : \text{AcOEt} = 5 : 1$ was collected. Further this fraction was rechromatographed (benzene/ AcOEt solvent system), and fraction eluted with benzene : $\text{AcOEt} = 10 : 1$ afforded squamostanal-A (**17**) (2 mg) as an oil. MF: 294, MW: $\text{C}_{18}\text{H}_{30}\text{O}_3$, State: oils, CD (MeOH) $\Delta\epsilon(\text{nm})$: -0.42 (240), HR-FAB-MS: 295.2225 (Calcd for $\text{C}_{18}\text{H}_{31}\text{O}_3$ 295.2273), $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ : 1.39 (3H, d, $J=6.8$ Hz, H-18), 2.42 (2H, td, $J=7.3, 1.9$ Hz, H-14), 2.26 (2H, t, $J=7.6$ Hz, H-3), 4.98 (1H, qq, $J=6.8, 1.5$ Hz, H-17), 6.98 (1H, d, $J=1.5$ Hz, H-16), 9.77 (1H, t, $J=1.9$ Hz, H-15), $^{13}\text{C-NMR}$ (67.5 MHz, CDCl_3) δ : 19.2 (C-18), 22.1 (C-13), 25.2 (C-3), 27.4 (C-4), 29-30 (C-6, -7, -8, -9, -10, -11, -12), 29.1 (C-5), 43.9 (C-14), 77.4 (C-17), 134.3 (C-2), 148.9 (C-16), 173.9 (C-1), 203.0 (C-15).

Squamocin-C

State: white crystals, MP: 50-51 °C. $[\alpha]_{\text{D}}^{25} +19.5$ ($c=0.92$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3690, 3585, 3460, 1750. UV (MeOH) λ_{max} nm(ϵ): 210 (7000). CD (MeOH) $\Delta\epsilon(\text{nm})$: -0.50 (240) HR-FAB-MS Calcd for $\text{C}_{37}\text{H}_{67}\text{O}_7$ (MH^+ ; m/z): 623.4887. Found: 623.4890. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.7$ Hz, H-34), 1.41 (3H, d, $J=7.1$ Hz, H-37), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.39 (1H, m, H-15), 3.58 (1H, m, H-29), 3.82-3.96 (5H, m, H-16, -19, -20, -23, -24), 4.99 (1H, q, $J=6.9$ Hz, H-36), 6.98 (1H, s, H-35), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.0 (C-34), 19.2 (C-37), 22.6 (C-33), 24.6 (C-22), 25.2 (C-3), 25.3 (C-31), 25.7 (C-13, -27), 26.1 (C-26), 27.4 (C-4), 28.4 (C-17), 28.9 (C-18, -21), 29.2 (C-5), 29-30 (C-6, -7, -8, -9, -10, -11, -12), 31.9 (C-32), 32.4 (C-25), 33.4 (C-14), 37.3 (C-30), 37.5 (C-28), 71.4 (C-24), 71.9 (C-29), 74.1 (C-15), 77.4 (C-36), 82.2 (C-19 or C-20), 82.5 (C-20 or C-19), 82.8 (C-23), 83.3 (C-16), 134.4 (C-2), 148.8 (C-35), 173.9 (C-1).

Squamocin-C triacetate

FAB-MS m/z 749(MH^+). $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.7$ Hz, H-34), 1.41 (3H, d, $J=7.1$ Hz, H-37), 2.04, 2.05, 2.08 (3H, each, s, AcO), 2.26 (2H, t, $J=7.4$ Hz, H-3), 3.90 (2H, m, H-19, -20), 3.98 (2H, m, H-16, -23), 4.82-4.92 (3H, m, H-15, -24, -29), 4.99 (1H, q, $J=7.1$ Hz, H-36), 6.99 (1H, s, H-35).

Tris-(*R*)-MTPA ester of squamocin-C

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.87 (3H, t, $J=7.0$ Hz, H-34), 1.40 (3H, d, $J=7.0$ Hz, H-37), 2.27 (2H, t, $J=6.8$ Hz, H-3), 3.52, 3.55, 3.61 (3H each, s, OMe), 3.66, 3.83 (1H each, m, H-19, -20), 3.90 (1H, m, H-23), 3.99 (1H, q, $J=7.3$ Hz, H-16), 4.99 (1H, qq, $J=6.6, 1.5$ Hz, H-36), 5.04 (2H, m, H-15, -29), 5.16 (1H, m, H-24), 6.98 (1H, s, H-35), 7.35-7.65 (15H, m, aromatic).

Squamocin-D

A colorless oil. $[\alpha]_{\text{D}}^{25} +30.1$ ($c=0.58$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3560, 3450, 1745. HR-FAB-MS Calcd for $\text{C}_{37}\text{H}_{67}\text{O}_7$ (MH^+ ; m/z): 623.4887. Found: 623.4830. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.1$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), 1.54 (2H, m, H-4), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.40 (2H, m, H-15, -24), 3.60 (1H, m, H-28), 3.81-3.93 (4H, m, H-16, -19, -20, -23), 4.99 (1H, q, $J=7.1$ Hz, H-36), 6.98 (1H, s, H-35), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.1 (C-34), 19.2 (C-37),

21.7 (C-26), 22.6 (C-33), 25.2 (C-3), 25.6 (C-13, -30), 27.4 (C-4), 28.4 (C-17, -22), 28.9 (C-18 or C-21), 29.0 (C-21 or C-18), 29.2 (C-5), 29.7 (C-31), 29-30 (C-6, -7, -8, -9, -10, -11, -12), 31.8 (C-32), 33.2 (C-25), 33.4 (C-14), 37.3 (C-27), 37.5 (C-29), 71.7 (C-28), 73.9 (C-24), 74.1 (C-15), 77.4 (C-36), 81.8 (C-19, -20), 83.1 (C-16 or C-23), 83.2 (C-23 or C-16), 134.3 (C-2), 148.8 (C-35), 173.9 (C-1).

Squamocin-D triacetate

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.6$ Hz, H-34), 1.41 (3H, d, $J=7.3$ Hz, H-37), 2.03 (3H, s, AcO), 2.07 (6H, s, AcO), 2.26 (2H, t, $J=7.5$ Hz, H-3), 3.90 (2H, m, H-19, -20), 3.98 (2H, q, $J=6.1$ Hz, H-16, -23), 4.81-4.88 (3H, m, H-15, -24, -28), 4.99 (1H, q, $J=7.0$ Hz, H-36), 6.99 (1H, s, H-35).

Tris-(*R*)-MTPA ester of squamocin-D

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.0$ Hz, H-34), 1.40 (3H, d, $J=7.0$ Hz, H-37), 2.26 (2H, t, $J=7.0$ Hz, H-3), 3.534 (3H, s, OMe), 3.587 (3H, s, OMe), 3.599 (3H, s, OMe), 3.88-3.96 (3H, m, H-19, -20, -23), 3.99 (1H, q, $J=7.1$ Hz, H-16), 4.90-4.98 (2H, m, H-24, -28), 4.99 (1H, q, $J=7.1$ Hz, H-36), 5.03 (1H, m, H-15), 6.98 (1H, s, H-35), 7.35-7.64 (15H, m, aromatic).

Squamocin-F

State: A white wax. $[\alpha]_{\text{D}}^{25} +21.0$ ($c=0.58$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3560, 3450, 1750. HR-FAB-MS Calcd for $\text{C}_{37}\text{H}_{67}\text{O}_7$ (MH^+ ; m/z): 623.4887. Found: 623.4890. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.1$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.40 (1H, m, H-24), 3.44 (1H, m, H-15), 3.60 (1H, m, H-12), 3.80-3.94 (4H, m, H-16, -19, -20, -23), 4.99 (1H, q, $J=7.1$ Hz, H-36), 6.99 (1H, s, H-35), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.2 (C-34), 19.3 (C-37), 22.8 (C-33), 25.1 (C-3), 25.6 (C-26), 25.7 (C-10), 27.4 (C-4), 28.3 (C-17 or C-22), 28.4 (C-22 or C-17), 28.9 (C-18, -21), 29.1 (C-5), 29-30 (C-6, -7, -8, -9, -14, -27, -28, -29, -30, -31), 32.0 (C-32), 33.4 (C-25), 33.5 (C-13), 37.6 (C-11), 71.7 (C-12), 74.0 (C-24), 74.2 (C-15), 77.5 (C-36), 81.7 (C-20 or C-19), 81.8 (C-19 or C-20), 83.2 (C-16, -23), 134.3, (C-2), 149.0 (C-36), 173.9 (C-1).

Squamocin-F triacetate

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.0$ Hz, H-34), 1.41 (3H, d, $J=6.4$ Hz, H-37), 2.036, 2.074, 2.077 (3H each, s, AcO), 2.26 (2H, t, $J=8.0$ Hz, H-3), 3.90 (2H, m, H-19, -20), 3.99 (2H, q, $J=4.8$ Hz, H-16, -23), 4.85 (3H, m, H-12, -15, -24), 4.99 (1H, qq, $J=6.7, 1.5$ Hz, H-36), 6.98 (1H, s, H-35).

Tris-(*R*)-MTPA ester of squamocin-F

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.1$ Hz, H-34), 1.40 (3H, d, $J=6.8$ Hz, H-37), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.481, 3.587, 3.593 (3H each, s, OMe), 3.89-3.94 (2H, m, H-19, -20), 3.94-4.02 (2H, m, H-16, -23), 4.90-5.05 (4H, m, H-12, -15, -24, -36), 6.98 (1H, s, H-35), 7.33-7.64 (15H, m, aromatic).

Squamocin-G (=bullatacin, rolliniastatin-2)

State: white crystals, MP: 77-78 (from MeOH-water). $[\alpha]_{\text{D}}^{25} +28.5$ ($c=0.50$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3580, 3450, 1750. CD (MeOH) $\Delta\epsilon(\text{nm})$: -40 (240). Anal, Found: C, 71.64; H, 10.64. Calcd for $\text{C}_{37}\text{H}_{66}\text{O}_7$: C, 71.34; H, 10.68. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.89 (3H, t, $J=6.9$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), 2.40 (1H, dd, $J=15.0, 8.2$ Hz, H-3a), 2.52 (1H, br d, $J=15.0$ Hz, H-3b), 3.38 (1H, m, H-15), 3.77-3.80 (3H, m, H-16, -23, -24), 3.80 (1H, m, H-4), 3.92 (2H, m, H-19, 20), 4.98 (1H, qq, $J=6.8, 1.4$ Hz, H-36), 6.98 (1H, br s, H-35), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.0 (C-34), 19.0 (C-37), 22.6 (C-33), 24.4 (C-22), 25.5 (C-6 or C-13), 25.7 (C-13 or C-6), 26.0 (C-26), 28.3 (C-17), 28.9 (C-18, -21), 29-30, (C-7, -8, -9, -10, -11, -12, -27,

-28, -29, -30, -31), 31.8 (C-32), 32.4 (C-25), 33.2 (C-3), 33.3 (-14), 37.3 (C-5), 70.0 (C-4), 71.3 (C-24), 74.2 (C-15), 77.9 (C-36), 82.3 (C-19 or C-20), 82.6 (C-20 or C-19), 83.3 (C-16), 131.1 (C-2), 151.7 (C-35), 174.5 (C-1).

Tris-(*R*)-MTPA ester of squamocin-G

¹H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, *J*=7.1 Hz, H-34), 1.41 (3H, d, *J*=7.0 Hz, H-37), 2.59 (1H, ddt, *J*=16.0, 3.8, 1.8 Hz, H-3a), 2.68 (1H, dd *J*=16.0, 7.2 Hz, H-3b), 3.502 (3H, s, OMe), 3.543 (3H, s, OMe), 3.612 (3H, s, OMe), 3.65 (1H, q, *J*=6.7 Hz, H-19 or -20), 3.83 (1H, m, H-20 or -19), 3.93 (1H, m, H-23), 4.00 (1H, q, *J*=7.4 Hz, H-16), 4.90 (1H, qq, *J*=6.9, 1.8 Hz, H-36), 5.03 (1H, q, *J*=6.7 Hz, H-15), 5.23 (1H, q, *J*=5.9 Hz, H-24), 5.38 (1H, m, H-4), 6.97 (1H, d, *J*=1.5 Hz, H-15, H-35), 7.34-7.66 (15H, m, aromatic).

Tris-(*S*)-MTPA ester of squamocin-G

¹H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, *J*=6.7 Hz, H-34), 1.28 (3H, d, *J*=7.2 Hz, H-37), 2.58 (2H, m, H-3), 3.519 (3H, s, OMe), 3.523 (3H, s, OMe), 3.549 (3H, s, OMe), 3.79 (2H, m, H-19, -20), 3.99 (1H, m, H-23), 4.03 (1H, q, *J*=8.7 Hz, H-16), 4.86 (1H, q, *J*=6.8 Hz, H-36), 5.06 (1H, q, *J*=6.4 Hz, H-15), 5.25 (1H, m, H-24), 5.32 (1H, br d, *J*=5.7 Hz, H-4), 6.73 (1H, d, *J*=1.5 Hz, H-35), 7.34-7.67 (15H, m, aromatic).

Squamocin-H(=asimicin)

State: white crystals, MP:45-48 (from MeOH-water). $[\alpha]_D^{25} +21.8$ (*c*=0.61, MeOH). Anal, Found: C, 71.33; H, 10.87. Calcd for C₃₇H₆₆O₇: C, 71.34; H, 10.68. ¹H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, *J*=6.9 Hz, H-34), 1.43 (3H, d, *J*=6.7 Hz, H-37), 2.40 (1H, dd, *J*=15.0, 8.2 Hz, H-3a), 2.53 (1H, br d, *J*=15.0 Hz, H-3b), 3.39 (2H, m, H-15, -24), 3.82-3.90 (5H, m, H-4, -16, -19, -20, -23), 5.06 (1H, qq, *J*=6.9, 1.4 Hz, H-36), 7.19 (1H, br s, H-35). ¹³C-NMR (125 MHz, CDCl₃) δ: 14.1 (C-34), 19.1 (C-37), 22.7 (C-33), 25.5 (C-6, -13), 25.6 (C-26), 28.3 (C-17, -22), 28.9 (C-18, -21), 29-30 (C-7, -8, -9, -10, -11, -12, -27, -28, -29, -30, -31), 31.9 (C-32), 33.3 (C-3), 33.5 (C-14, -25), 37.3 (C-5), 70.0 (C-4), 74.1 (C-15, -24), 77.9 (C-36), 81.7 (C-19, -20), 83.1 (C-16, -23), 131.2 (C-2), 151.7 (C-35), 174.6 (C-1).

Squamocin-I

State: white needles, MP: 68.5-71 (from MeOH-water). $[\alpha]_D^{25} +22.2$ (*c*=0.50, MeOH). IR ν_{\max} (CHCl₃) cm⁻¹: 3560, 3450, 1750. UV λ_{\max} (MeOH) nm (log ϵ): 210 (3.7). CD (MeOH) $\Delta\epsilon$ (nm): -0.29 (239) HR-FAB-MS Calcd for C₃₅H₆₃O₆ (MH⁺; *m/z*): 579.4625. Found: 579.4640. ¹H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, *J*=6.9 Hz, H-32), 1.41 (3H, d, *J*=6.9 Hz, H-35), 2.26 (2H, t, *J*=7.7 Hz, H-3), 3.40 (1H, m, H-22), 3.80-3.90 (3H, m, H-13, 14, 21), 3.90-3.97 (2H, m, H-17, -18), 5.00 (1H, q, *J*=7.1 Hz, H-34), 6.98 (1H, br s, H-33), ¹³C-NMR (125 MHz, CDCl₃) δ: 14.1 (C-32), 19.2 (C-35), 22.6 (C-31), 24.4 (C-15), 25.2 (C-3), 25.5 (C-24), 26.0 (C-11), 27.3 (C-4), 28.4 (C-20), 28.9 (C-16, -19), 29.1 (C-5), 29-30 (C-6, -7, -8, -9, -10, -25, -26, -27, -28, -29), 31.9 (C-30), 32.4 (C-12), 33.2 (C-23), 71.3 (C-13), 74.1 (C-22), 77.3 (C-34), 82.2 (C-18 or C-17), 82.5 (C-17 or C-18), 82.8 (C-14), 83.3 (C-21), 134.3 (C-2), 148.8 (C-33), 173.8 (C-1).

Squamocin-I diacetate

¹H-NMR δ: 0.88 (3H, t, *J*=6.9 Hz, H-32), 1.40 (3H, d, *J*=6.6 Hz, H-35), 2.045 (3H, s, AcO), 2.074 (3H, s, AcO), 2.26 (2H, t, *J*=7.7 Hz, H-3), 3.89 (2H, m, H-17, -18), 3.98 (2H, qui, *J*=6.8 Hz, H-14, -21), 4.86 (1H, m, H-22), 4.91 (1H, m, H-13), 4.99 (1H, q, *J*=6.6 Hz, H-34), 6.98 (1H, br s, H-33).

Di-(*R*)-MTPA ester of squamocin-I

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.0$ Hz, H-32), 1.40 (3H, d, $J=7.0$ Hz, H-35), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.543 (3H, s, OMe), 3.611 (3H, s, OMe), 3.65 (1H, m, H-17 or H-18), 3.83 (1H, m, H-18 or H-17), 3.93 (1H, m, H-14), 4.00 (1H, q, $J=7.0$ Hz, H-21), 4.99 (1H, q, $J=7.0$ Hz, H-34), 5.03 (1H, q, $J=7.8$ Hz, H-22), 5.23 (1H, q, $J=6.0$ Hz, H-13), 6.98 (1H, d, $J=1.5$ Hz, H-33), 7.34-7.65 (10H, m, aromatic).

Squamocin-J

State: white needles, MP: 85-86.5 (from MeOH-water). $[\alpha]_{\text{D}}^{25} +18.6$ ($c=0.42$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3560, 3450, 1750. UV λ_{max} (MeOH) nm (log ϵ): 209 (3.8). CD (MeOH) $\Delta\epsilon(\text{nm})$: -0.40 (241), Anal, Found: C, 73.08; H, 10.72. Calcd for $\text{C}_{35}\text{H}_{62}\text{O}_6$: C, 72.62; H, 10.80. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.9$ Hz, H-32), 1.41 (3H, d, $J=6.9$ Hz, H-35), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.40 (1H, m, H-13), 3.82-3.90 (3H, m, H-14, 21, -22), 3.90-3.97 (2H, m, H-17, -18), 5.00 (1H, q, $J=7.0$ Hz, H-34), 6.99 (1H, br s, H-33), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.1 (C-32), 19.2 (C-35), 22.7 (C-31), 24.5 (C-20), 25.2 (C-3), 25.7 (C-11), 26.0 (C-24), 27.4 (C-4), 28.4 (C-15), 28.9 (C-16), 29.0 (C-19), 29.2 (C-5), 29-30 (C-6, -7, -8, -9, -10, -25, -26, -27, -28, -29), 31.9 (C-30), 32.5 (C-23), 33.4 (C-12), 71.4 (C-22), 74.1 (C-13), 77.4 (C-34), 82.3 (C-17 or C-18), 82.5 (C-18 or C-17), 82.8 (C-21), 83.3 (C-14), 134.3 (C-2), 148.8 (C-33), 173.9 (C-1).

Squamocin-J diacetate

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.9$ Hz, H-32), 1.41 (3H, d, $J=6.6$ Hz, H-35), 2.047 (3H, s, AcO), 2.074 (3H, s, AcO), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.89 (2H, m, H-17, -18), 3.98 (2H, qui, $J=6.9$ Hz, H-14, -21), 4.86 (1H, m, H-13), 4.91 (1H, m, H-22), 4.99 (1H, q, $J=6.8$ Hz, H-34), 6.98 (1H, br s, H-33).

Di-(*R*)-MTPA ester squamocin-J

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.3$ Hz, H-32), 1.40 (3H, d, $J=6.5$ Hz, H-35), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.543 (3H, s, OMe), 3.621 (3H, s, OMe), 3.65 (1H, m, H-18 or H-17), 3.83 (1H, m, H-17 or H-18), 3.93 (1H, m, H-21), 4.00 (1H, q, $J=7.4$ Hz, H-14), 4.99 (1H, q, $J=7.0$ Hz, H-34), 5.03 (1H, q, $J=7.0$ Hz, H-13), 5.23 (1H, q, $J=6.5$ Hz, H-22), 6.98 (1H, d, $J=1.5$ Hz, H-33), 7.34-7.65 (10H, m, aromatic).

Squamocin-K

State: a white wax, $[\alpha]_{\text{D}}^{25} +20.5$ ($c=0.53$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3560, 3450, 1750. Anal, Found: C, 72.34; H, 10.96. Calcd for $\text{C}_{35}\text{H}_{62}\text{O}_6$: C, 72.62; H, 10.80. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.9$ Hz, H-32), 1.40 (3H, d, $J=6.7$ Hz, H-35), 2.26 (2H, t, $J=8.0$ Hz, H-3), 3.39 (2H, m, H-13, -22), 3.81-3.87 (2H, q, $J=6.8$ Hz, H-14, 21), 3.87-3.93 (2H, m, H-17, -18), 4.99 (1H, q, $J=6.8$ Hz, H-34), 6.98 (1H, d, $J=1.4$ Hz, H-33), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.0 (C-32), 19.2 (C-35), 22.6 (C-31), 25.1 (C-3), 25.6 (C-11, -24), 27.3 (C-4), 28.4 (C-15, -20), 28.9 (C-16, -19), 29.1 (C-5), 29-30 (C-6, -7, -8, -9, -10, -25, -26, -27, -28, -29), 31.9 (C-30), 33.4 (C-12, -23), 74.0 (C-13, -22), 77.3 (C-34), 81.7 (C-17, -18), 83.1 (C-14, -21), 134.3 (C-2), 148.8 (C-33), 173.8 (C-1).

Squamocin-K diacetate

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.0$ Hz, H-32), 1.40 (3H, d, $J=7.1$ Hz, H-35), 2.074 (6H, s, AcO), 2.26 (2H, t, $J=8.0$ Hz, H-3), 3.91 (2H, m, H-17, -18), 3.99 (2H, m, H-14, -21), 4.86 (2H, m, H-13, -22), 4.99 (1H, q, $J=6.8$ Hz, H-34), 6.98 (1H, br s, H-33).

Di-(*R*)-MTPA ester of squamocin-K

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.0$ Hz, H-32), 1.40 (3H, d, $J=7.0$ Hz, H-35), 2.26 (2H, t, $J=8.0$ Hz, H-3), 3.603 (3H, s, OMe), 3.606 (3H, s, OMe), 3.93 (2H, m, H-17, -18), 4.00 (2H, q, $J=7.0$ Hz, H-14, -21), 4.99 (1H, q, $J=6.8$ Hz, H-34), 5.03 (1H, q, $J=7.5$ Hz, H-13, -22), 6.98 (1H, d, $J=1.5$ Hz, H-33), 7.34-7.66 (10H, m, aromatic).

Squamocin-L

State: white crystals, MP: 67.5-69 (from MeOH-water). $[\alpha]_{\text{D}}^{25} +19.3$ ($c=0.98$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3590, 3450, 1745. Anal, Found: C, 73.01; H, 11.25. Calcd for $\text{C}_{37}\text{H}_{66}\text{O}_6$: C, 73.22; H, 10.96. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.9$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), 2.26 (2H, t, $J=6.7$ Hz, H-3), 3.40 (1H, m, H-15), 3.81-3.90 (3H, m, H-16, -23, -24), 3.91-3.98 (2H, m, H-19, 20), 5.00 (1H, q, $J=7.0$ Hz, H-36), 6.99 (1H, br s, H-35), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.0 (C-34), 19.2 (C-37), 22.6 (C-33), 24.4 (C-22), 25.1 (C-3), 25.6 (C-13), 26.0 (C-26), 27.4 (C-4), 28.4 (C-17), 29.0 (C-18), 29.1 (C-5, -21), 29-30 (C-6, -7, -8, -9, -10, -11, -12, -27, -28, -29, -30, -31), 31.9 (C-32), 32.4 (C-25), 33.3 (C-14), 71.5 (C-24), 74.1 (C-15), 77.3 (C-36), 82.2 (C-19 or C-20), 82.5 (C-20 or C-19), 82.8 (C-23), 83.3 (C-16), 134.3 (C-2), 148.8 (C-35), 173.8 (C-1).

Di-(*R*)-MTPA ester of squamocin-L

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.8$ Hz, H-34), 1.41 (3H, d, $J=6.8$ Hz, H-37), 2.27 (2H, t, $J=7.6$ Hz, H-3), 3.545 (3H, s, OMe), 3.617 (3H, s, OMe), 3.64 (1H, m, H-19 or -20), 3.83 (1H, m, H-20 or -19), 3.94 (1H, m, H-23), 4.00 (1H, q, $J=6.8$ Hz, H-16), 5.02 (2H, m, H-15, -36), 5.24 (1H, q, $J=6.0$ Hz, H-24), 6.98 (1H, s, H-35), 7.35-7.68 (15H, m, aromatic).

Squamocin-M

State: a colorless oil. $[\alpha]_{\text{D}}^{25} +26.0$ ($c=0.55$, MeOH). Anal, Found: C, 73.01; H, 11.25. Calcd for $\text{C}_{37}\text{H}_{66}\text{O}_6$: C, 73.22; H, 10.96. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.9$ Hz, H-34), 1.40 (3H, d, $J=6.9$ Hz, H-37), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.39 (2H, m, H-15, -24), 3.81-3.87 (2H, q, $J=6.0$ Hz, H-16, -23), 3.87-3.93 (2H, m, H-19, 20), 4.99 (1H, q, $J=6.8$ Hz, H-36), 6.98 (1H, d, $J=1.4$ Hz, H-35). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.1 (C-34), 19.2 (C-37), 22.0 (C-26), 22.6 (C-33), 25.2 (C-3), 25.4 (C-22), 25.7 (C-30), 26.1 (C-10), 27.4 (C-4), 28.4 (C-14), 28.6 (C-21), 29.7 (C-31), 29-31 (C-5, -6, -7, -8, -9, -17, -18), 31.8 (C-32), 32.4 (C-13), 32.5 (C-25), 35.6 (C-11), 37.3 (C-27), 37.5 (C-29), 71.6 (C-24), 71.8 (C-28), 74.5 (C-16 or -19), 74.6 (C-19 or -16), 77.4 (C-36), 79.3 (C-12), 82.0 (C-15), 82.2 (C-23), 83.4 (C-20), 134.3 (C-2), 148.9 (C-35), 173.9 (C-1).

Di-(*R*)-MTPA ester of squamocin-M

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.7$ Hz, H-34), 1.40 (3H, d, $J=6.8$ Hz, H-37), 2.26 (2H, t, $J=7.3$, 1.4 Hz, H-3), 3.606 (6H, s, OMe), 3.93 (2H, m, H-19, -20), 3.99 (2H, q, $J=6.6$ Hz, H-16, -23), 5.01 (3H, m, H-15, -24, -36), 6.98 (1H, d, $J=1.5$ Hz, H-35), 7.32-7.67 (15H, m, aromatic).

Squamostatin-A

State: white crystals, MP: 87-89 (from AcOEt). $[\alpha]_{\text{D}}^{25} +11.0$ ($c=0.40$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3680, 3590, 3400, 1750. UV λ_{max} (MeOH) nm (ϵ): 210 (9300). CD (MeOH) $\Delta\epsilon(\text{nm})$: -0.50 (240), $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.89 (3H, t, $J=5.9$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), 2.26 (2H, tt, $J=7.7$, 1.4 Hz, H-3), 3.41 (2H, m, H-16, -19), 3.77-3.90 (5H, m, H-12, -15, -20, -23, -24), 4.99 (1H, qq, $J=6.8$, 1.4 Hz, H-36), 6.98 (1H, br s, H-35), $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.1 (C-34), 19.2 (C-37), 22.0 (C-26), 22.6 (C-33), 25.2 (C-3), 25.4 (C-22), 25.7 (C-30), 26.1 (C-10), 27.4 (C-4), 28.4 (C-14), 28.6 (C-21), 29.7 (C-31), 29-31 (C-5, -6, -7, -8, -9, -17, -18), 31.8 (C-32), 32.4 (C-13), 32.5 (C-25), 35.6 (C-11), 37.3 (C-27), 37.5 (C-29), 71.6 (C-24), 71.8 (C-28), 74.5 (C-16 or -19), 74.6 (C-19 or -16), 77.4 (C-36), 79.3 (C-12), 82.0 (C-15), 82.2 (C-23), 83.4 (C-20), 134.3 (C-2), 148.9 (C-35), 173.9 (C-1).

Tetra-(*R*)-MTPA ester of squamostatin-A

¹H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, *J*=6.9 Hz, H-34), 1.41 (3H, d, *J*=7.0 Hz, H-37), 2.26 (2H, tt, *J*=7.7, 1.4 Hz, H-3), 3.471 (3H, s, OMe), 3.490 (3H, s, OMe), 3.535 (3H, s, OMe), 3.578 (3H, s, OMe), 3.67 (1H, m, H-20), 3.74 (1H, m, H-12), 3.89 (2H, m, H-15, -23), 4.91 (2H, m, H-16, -19), 4.98 (1H, qq, *J*=6.8, 1.4 Hz, H-36), 5.01 (1H, qui, *J*=6.0 Hz, H-28), 5.16 (1H, q, *J*=5.8 Hz, H-24), 6.98 (1H, br s, H-35), 7.30-7.65 (20H, m, aromatic).

Squamostatin-B

State: white crystals, MP: 98-101 (from AcOEt). $[\alpha]_D^{25} +10.5$ (*c*=0.10, MeOH). IR ν_{\max} (CHCl₃) cm⁻¹: 3590, 3450, 1745. UV λ_{\max} (MeOH) nm (ϵ): 209 (7000). CD (MeOH) $\Delta\epsilon$ (nm): -0.50 (238), HR-FAB-MS Calcd for C₃₇H₆₇O₈ (MH⁺; *m/z*): 639.4836. Found: 639.4890. ¹H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, *J*=6.8 Hz, H-34), 1.43 (3H, d, *J*=6.8 Hz, H-37), 2.40 (1H, ddt, *J*=15.0, 8.3, 1.5 Hz, H-3a), 2.53 (1H, ddt, *J*=15.0, 3.0, 1.5 Hz, H-3b), 3.41 (2H, m, H-16, -19), 3.76-3.91 (6H, m, H-4, -12, -15, -20, -23, -24), 5.06 (1H, q, *J*=6.8 Hz, H-36), 7.19 (1H, s, H-35). ¹³C-NMR (125 MHz, CDCl₃) δ: 14.1 (C-34), 19.1 (C-37), 22.7 (C-33), 25.2 (C-22), 25.5 (C-6), 26.0 (C-26), 26.2 (C-10), 28.4 (C-14), 28.6 (C-21), 29-31 (C-7, -8, -9, -17, -18, -27, -28, -29, -30, -31), 31.9 (C-32), 32.4 (C-13), 32.6 (C-25), 33.4 (C-3), 35.6 (C-11), 37.4 (C-5), 70.0 (C-4), 71.6 (C-24), 74.5 (C-16 or -19), 74.6 (C-19 or -16), 78.0 (C-36), 79.3 (C-12), 82.0 (C-15), 82.2 (C-23), 83.3 (C-20), 131.2 (C-2), 151.8 (C-35), 174.6 (C-1).

Tetra-(*R*)-MTPA ester of squamostatin-B

¹H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, *J*=7.0 Hz, H-34), 1.31 (3H, d, *J*=6.6 Hz, H-37), 2.59 (1H, ddt, *J*=15.6, 2.0, 2.0 Hz, H-3a), 2.67 (1H, dd, *J*=15.6, 7.8 Hz, H-3b), 3.470 (3H, s, OMe), 3.495 (3H, s, OMe), 3.523 (3H, s, OMe), 3.580 (3H, s, OMe), 3.69 (1H, q, *J*=7.5 Hz, H-20), 3.74 (1H, m, H-12), 3.88 (1H, q, *J*=6.9 Hz, H-15), 3.97 (1H, m, H-4), 4.88-4.94 (3H, m, H-16, -19, -36), 5.26 (1H, m, H-24), 5.37 (1H, m, H-4), 6.96 (1H, s, H-35), 7.30-7.65 (20H, m, aromatic).

Squamostatin-C

State: white crystals, MP: 95-97 (from AcOEt). $[\alpha]_D^{25} +12.0$ (*c*=0.10, MeOH). IR ν_{\max} (CHCl₃) cm⁻¹: 3685, 3585, 3540, 1755. UV λ_{\max} (MeOH) nm (ϵ): 210 (7000). CD (MeOH) $\Delta\epsilon$ (nm): -0.50 (238), HR-FAB-MS Calcd for C₃₇H₆₇O₈ (MH⁺; *m/z*): 639.4836. Found: 639.4890. ¹H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, *J*=7.1 Hz, H-34), 1.43 (3H, d, *J*=6.8 Hz, H-37), 2.40 (1H, ddt, *J*=15.0, 8.2, 1.6 Hz, H-3a), 2.53 (1H, ddt, *J*=15.0, 4.0, 2.0 Hz, H-3b), 3.41 (3H, m, H-16, -19, -24), 3.77-3.90 (5H, m, H-4, -12, -15, -20, -23), 5.06 (1H, q, *J*=6.8 Hz, H-36), 7.19 (1H, s, H-35). ¹³C-NMR (125 MHz, CDCl₃) δ: 14.1 (C-34), 19.1 (C-37), 22.7 (C-33), 25.5 (C-6), 25.6 (C-26), 26.1 (C-10), 28.4 (C-14), 28.7 (C-21, -22), 29-31 (C-7, -8, -9, -17, -18, -27, -28, -29, -30, -31), 31.9 (C-32), 32.4 (C-13), 33.4 (C-3), 33.5 (C-25), 35.6 (C-11), 37.4 (C-5), 70.0 (C-4), 74.0 (C-24), 74.3 (C-19 or -16), 74.4 (C-16 or -19), 77.9 (C-36), 79.3 (C-12), 82.0 (C-15), 82.7 (C-20, -23), 131.2 (C-2), 151.7 (C-35), 174.6 (C-1).

Tetra-(*R*)-MTPA ester of squamostatin-C

¹H-NMR (500 MHz, CDCl₃) δ: 0.89 (3H, t, *J*=7.0 Hz, H-34), 1.31 (3H, d, *J*=6.8 Hz, H-37), 2.59 (1H, ddt, *J*=15.6, 2.0, 2.0 Hz, H-3a), 2.67 (1H, dd, *J*=15.6, 7.8 Hz, H-3b), 3.466 (3H, s, OMe), 3.495 (3H, s, OMe), 3.507 (3H, s, OMe), 3.530 (3H, s, OMe), 3.74 (1H, m, H-12), 3.87 (1H, m, H-15), 3.93 (1H, m, H-20), 4.02 (1H, m, H-23), 4.90 (2H, m, H-16, -36), 4.99 (1H, m, H-19), 5.02 (1H, m, H-24), 5.37 (1H, m, H-4), 6.96 (1H, s, H-35), 7.30-7.64 (20H, m, aromatic).

Squamostatin-D

State: white crystals, MP: 112-113.5 (from MeOH-H₂O). $[\alpha]_D^{25} +7.9$ (*c*=0.51, MeOH). IR ν_{\max} (CHCl₃) cm⁻¹: 3560, 3450, 1755. HR-FAB-MS Calcd for C₃₇H₆₇O₇ (MH⁺; *m/z*): 623.4887. Found: 623.4882. ¹H-NMR (500 MHz, CDCl₃) δ: 0.89

(3H, t, $J=5.9$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), 2.26 (2H, tt, $J=7.7, 7.1$ Hz, H-3), 3.41 (2H, m, H-16, -19), 3.77-3.90 (5H, m, H-12, -15, -20, -23, -24), 4.99 (1H, qq, $J=6.8, 1.4$ Hz, H-36), 6.98 (1H, s, H-35). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.1 (C-34), 19.2 (C-37), 22.7 (C-33), 25.2 (C-3, -22), 26.0 (C-26), 26.2 (C-10), 27.4 (C-4), 28.4 (C-14), 28.6 (C-21), 29-31 (C-5, -6, -7, -8, -9, -17, -18, -27, -28, -29, -30, -31), 31.9 (C-32), 32.4 (C-13), 32.5 (C-25), 35.6 (C-11), 71.5 (C-24), 74.5 (C-16 or -19), 74.6 (C-19 or -16), 77.4 (C-36), 79.3 (C-12), 82.0 (C-15), 82.2 (C-23), 83.3 (C-20), 134.3 (C-2), 148.8 (C-35), 173.9 (C-1).

Tris-(*R*)-MTPA ester of squamostatin-D

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.0$ Hz, H-34), 1.40 (3H, d, $J=7.0$ Hz, H-37), 2.26 (2H, tt, $J=7.7, 7.1$ Hz, H-3), 3.474 (3H, s, OMe), 3.525 (3H, s, OMe), 3.582 (3H, s, OMe), 3.69 (1H, q, $J=7.5$ Hz, H-20), 3.75 (1H, m, H-12), 3.89 (1H, q, $J=7.0$ Hz, H-15), 3.97 (1H, m, H-23), 4.91 (2H, m, H-16, -36), 4.99 (1H, qq, $J=6.8, 1.4$ Hz, H-36), 5.26 (1H, m, H-24), 6.97 (1H, d, $J=1.5$ Hz, H-35), 7.33-7.63 (20H, m, aromatic).

Squamostatin-E

State: white crystals, MP: 105-106 (from MeOH-H₂O). $[\alpha]_D^{25} +14.7$ ($c=0.51$, MeOH). IR ν_{max} (CHCl_3) cm^{-1} : 3560, 3450, 1750. Anal, Found: C, 71.64; H, 10.98. Calcd for $\text{C}_{37}\text{H}_{66}\text{O}_7$: C, 71.34; H, 10.68. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=6.7$ Hz, H-34), 1.42 (3H, d, $J=6.7$ Hz, H-37), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.38-3.57 (3H, m, H-16, -19, -24), 3.77-3.92 (4H, m, H-12, -15, -20, -23), 4.98 (1H, qq, $J=6.8, 1.4$ Hz, H-36), 6.98 (1H, br s, H-35). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 14.1 (C-34), 19.2 (C-37), 22.6 (C-33), 25.1 (C-3), 25.6 (C-26), 26.2 (C-10), 27.4 (C-4), 28.4 (C-14), 28.7 (C-21, -22), 29-31 (C-5, -6, -7, -8, -9, -17, -18, -27, -28, -29, -30, -31), 31.9 (C-32), 32.4 (C-13), 33.4 (C-25), 35.6 (C-11), 74.1 (C-24), 74.2 (C-19 or -16), 74.4 (C-16 or 19), 77.4 (C-36), 79.3 (C-12), 82.0 (C-15), 82.7 (C-20, -23), 134.3 (C-2), 148.8 (C-35), 173.9 (C-1).

Tris-(*R*)-MTPA ester of squamostatin-E

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 0.88 (3H, t, $J=7.0$ Hz, H-34), 1.40 (3H, d, $J=6.7$ Hz, H-37), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.469 (3H, s, OMe), 3.508 (3H, s, OMe), 3.531 (3H, s, OMe), 3.74 (1H, m, H-12), 3.87 (1H, q, $J=7.0$ Hz, H-15), 3.93 (1H, q, $J=7.0$ Hz, H-20), 4.03 (1H, m, H-23), 4.91 (1H, q, $J=6.5$ Hz, H-16), 4.96-5.05 (3H, m, H-19, -24, -36), 6.97 (1H, d, $J=1.4$ Hz, H-35), 7.33-7.62 (20H, m, aromatic).

Inductions of callus from leaves of annonaceous plants (Dodds and Roberts, 1982)

Callus was induced according to a conventional method as given bellow.

- (1) Leaves washed with water were sunken in 70% ethanol solution for removal of wax, and these are rinsed with sterile water sufficiently.
- (2) After submerging of the leaves in a saturated chlorinated lime for ten minutes, they are washed with sterile water throughly.
- (3) The leaves were cut sterilized, and inoculated to petri dishes holded Murashige & Skoog medium (Murashige and Skoog, 1962) containing 2 mg/dm³ 2,4-dichlorophenoxyacetic acid.
- (4) These dishes were incubated at 25 under dark for 30-40 days.
- (5) The growing callus (about 5 mm diameter) was inoculated to petri dishes holded Nitsch & Nitsch (Nitsch and Nitsch, 1967) containing indole-3-acetic acid.
- (6) Upon ubsequent inoculations, callus were cut to 5 mm diameter to petri dishes holded Nitsch & Nitsch containing indole-3-acetic acid.

In case of *A. cherimolia*, induction of callus and subsequent culture were succeeded. The growing speed of callus

was influenced by dose of indole-3-acetic acid to the medium. It was taken about 40 days that 5 mm diameter callus grew to >10 mm callus.

In case of *A. squamosa* L., induction of callus (about 3 mm diameter) was succeeded, but subsequent culture was not successful.

Preparation of medium for callus induction (Nair et al., 1983; 1984)

Murashige and Skoog medium (Murashige and Skoog, 1962) without sucrose, indole-3-acetic acid (Wako Pure Chemical) 5 mg, and Kinetin (ICN Biomedical) 4 mg, sucrose (Kanto Chemical) 30 g, gellan gum (Sanko Chemical) 2 g, 2,4-dichlorophenoxyacetic acid (Tokyo Kasei) 1 mg were dissolved to distilled water of 1 dm³ and prepared to pH 6.0. The solution was autoclaved at 121 °C, 15 min, and was pipette out to petri dishes or test tube for plant cell culture.

Preparation of medium (1) for maintenance of callus

Nitsch and Nitsch basal salt mixture (Nitsch and Nitsch, 1967) (SIGMA) 2.1 g, sucrose (Kanto Chemical) 15-60 g, gellan gum (Sanko Chemical) 2 g, indole-3-acetic acid (Wako Pure Chemical) 2-5 mg were dissolved to distilled water of 1 dm³ and prepared to pH 5.8-6.0. The solution was autoclaved at 121 °C, 15 min, and was pipette out to petri dishes or test tube for plant cell culture.

Preparation of medium (2) for maintenance of callus

Murashige and Skoog medium (Murashige and Skoog, 1962) without sucrose, sucrose (Kanto Chemical) 15-60 g, gellan gum (Sanko Chemical) 2 g, indole-3-acetic acid (Wako Pure Chemical) 2-5 mg were dissolved into distilled water of 1 dm³ and prepared to pH 5.8-6.0. The solution was autoclaved at 121 °C, 15 min, and was pipette out to petri dishes or test tube for plant cell culture.

Bioassay for inhibitory activity on lettuce seedling growth

A method of D. Aspinall et al. (1967) was modified. That is, aliquots of chloroform solution of acetogenins were applied separately on 4 cm diameter discs of ADVANTEC No. 1 filter paper in 4.0 cm i.d. petri dishes with a micropipette. The solvent was removed using an aspirator. The disks were wetted with 0.6 ml distilled water. Lettuce seeds (*Lactuca sativa* cv. Great Lakes 366) were obtained from Takii Seeds Co. Twelve seeds were placed on each paper disc. Germination was carried out in a moisture-saturated dark chamber for 72 hrs at 25 °C. Results were taken by measuring the length of the radicle and hypocotyl. The effects of radicle and hypocotyl exudates were expressed as percentage of inhibition relative to a control.

Acknowledgements

Since beginning of investigation about the organic chemistry, the author have a long list of people he want to thank who have helped him in many ways at this time. First, the author want to thank two people, Dr. Yoshinori Fujimoto, professor of Tokyo Institute of Technology, Dr. Hideo Inatomi and Dr. Tamiji Sugiyama, professors of Meiji University, for their guidance, practical advices and indispensable encouragement during the past decade or so with enormous enthusiasm, patience, and encouragement. The author would like to thank following people who have helped him during the completion of this thesis:

To Dr. Kazuo Hirayama of Ajinomoto Co. Ltd. for the discussions about MS analysis and technical assistants (derivatization with amine at gas phase, and measurement of the precursor ion scanning).

To Dr. Isao Kitagawa for the ionophore assay, and Dr. Mitsuji Yoshida for the cytotoxic assay.

To Mr. Noriyuki Hara and all members of Fujimoto group for their helpful discussions, inspiration and technical assistants.

To Dr. Yoshiharu Fujii and Dr. Syuntato Hiradate of National Institute for Agro-Environmental Sciences JAPAN, for their encouragement and comprehension of this work.

To Dr. Zahida Iqbal and Dr. Tetsuo Kushiro for a correction of English in the thesis.

Finally, the author is very grateful to his parents for their encouragement, support and patience.

This report is based on doctoral thesis of Hiroshi Araya and modified from it.

Appendix A

List of Abbreviations

Ac₂O: acetic anhydride

AcOEt: ethyl acetate

AcOH: acetic acid

CC: column chromatography

CD: circular dichroism

CI: chemical ionization

COSY: correlation spectroscopy

EI: electron ionization

er. erythro

FAB: fast atom bombardment

GC: gas chromatography

h: hour

HMBC: ¹H-detected multiple-bond heteronuclear multiple quantum coherence spectrum

HPLC: high performance liquid chromatography

HR: high resolution

INEPT: insensitive nucleic enhanced by polarization transfer

IR: infra red

m-CPBA: 3-chloroperoxybenzoic acid

Me: methyl

MeOH: methanol

MF: molecular formula
MW: molecular weight
min: minute(s)
ml: milliliter(s)
MP: melting point
MS: mass spectrometry
MTPA: α -methoxy- α -(trifluoromethyl)phenylacetyl
NMR: nuclear magnetic resonance
NOE: nuclear overhauser effect
ODS: octadecyl silyl
pet.: petroleum
p-TLC: preparative TLC
sq: squamocin
st: squamostatin
thr. threo
THF: tetrahydrofuran
TLC: thin layer chromatography
TMS: trimethylsilyl, tetramethylsilane
tr. trans
UV: ultra violet

Appendix B

List of Figures

1. *Annona squamosa* L.
2. Terpenoids and alkaloids found in annonaceae
3. Structure of uvaricin (1)
4. Classification of tetrahydrofuran portion
5. Structures of lactone moiety
6. Structures of squamocin (2) and squamostatin-A (3)
7. Difference between C1 and C2 in ^{13}C -NMR
8. Structure of goniocin from *Goniothalamus giganteus* bark
9. Examples of acetogenins of type (E)
10. EI-, CI- and FAB-MS spectra of squamocin (2)
11. FAB-MS spectrum of squamocin acetate
12. The image of product and precursor ion scanning method
13. Product ion scanning from m/z 623 for suamocin (2)
14. Precursor ion scanning from m/z 97 for squamocin (2)
15. Formation of m/z 97 fragment ion
16. Example of the derivatizaion for mass analysis of peptides

17. Predictable reaction of α,β -unsaturated- γ -lactone with amines
18. Structure of benzylamine derivative (**4**)
19. FAB-MS spectrum of the benzylamine derivative
20. Precursor ion scanning from m/z 91 for the benzylamine (**4**)
21. Expected fragment ions of *N,N*-dimethylethylenediamine derivative in FAB-MS
22. Two dimensional TLC experiment of derivative (**5**)
23. Two dimensional TLC experiment of crude reaction mixture
24. Structure of the amine derivative (**5**)
25. Structure of the amine derivative (**6**)
26. The FAB-MS spectrum of the amine derivative (**5**)
27. Precursor ion scanning from m/z 72 for the amine derivative (**5**)
28. Precursor ion scanning from m/z 72 for amine derivative of squamocin-C
29. Precursor ion scanning from m/z 72 for amine derivative of squamocin-F
30. EI-MS spectrum of squamostatin-C (**9**)
31. EI-MS fragment pattern of 4-hydroxy-squamocin-F
32. Precursor ion scanning from m/z 72 for amine derivative of squamostatin-C
33. EI-MS spectrum of annonacin-10-one (**10**)
34. Precursor ion scanning from m/z 72 for amine derivative of annonacin-10-one
35. Isolated tetrahydrofuranic acetogenins in this study
36. Isolation of new tetrahydrofuranic acetogenins
37. EI-MS fragment pattern of **11**
38. Precursor ion scanning of amination derivative (**11a**) from m/z 72
39. Chemical structures of squamocins-O₁ (**11**) and -O₂ (**12**)
40. Possible structure for squamosten-A (**13**)
41. Precursor ion scanning from m/z 309 for squamosten-A (**13**)
42. HPLC analysis of fatty acid *p*-bromophenacyl ester
43. Mosher's model for (*R*)- and (*S*)-MTPA esters
44. EI-MS fragmentation pattern of squamocin-N (**14**)
45. EI-MS fragmentation pattern of squamocin-E (**15**)
46. EI-MS fragmentation pattern of squamocin-B (**16**)
47. EI-MS fragmentation pattern of squamostanal-A (**17**)
48. Proposed reaction mechanism of γ -lactone portion with amine
49. Difference between product ion scanning based on charge remote fragmentation and precursor ion scanning based on the concept of "tag" (newly developed amine method)
50. Semi-synthesis of mono-tetrahydrofuranic acetogenins
51. Proposed biosynthetic pathway of tetrahydrofuran moiety *Annona squamosa* L. seeds
52. Biosynthetic pathway of acaterin
53. Inhibitory activity of annonaceous tetrahydrofuranic acetogenins to lettuce seedling growth

Appendix C

List of Tables

1. Genera of source of tetrahydrofuranic acetogenins
2. Hoye's rule: chemical shifts of diagnostic protons of model bis-tetrahydrofuran diacetates
3. Born's rule: chemical shifts of diagnostic protons and carbons of model tetrahydrofurans
4. NMR data of benzylamine derivative (**4**)
5. NMR data of the amine derivative (**5**)
6. NMR data of the amine derivative (**6**)
7. ¹³C-NMR spectral data for squamocins-O₁ (**11**) and -O₂ (**12**)
8. ¹H-NMR spectral data for MTPA esters (**11rs**, **12rs** and **2rs**)
9. Partial NMR δ value of MTPA esters (**11r**, **12r**, **18r** and **19r**)
10. Physico-chemical properties of squamosten-A (**13**)
11. ¹³C-NMR comparison of the signals of tetrahydrofuran moiety
12. ¹H-NMR data of (*R*)- and (*S*)-MTPA ester of squamocin-G and (*R*)-MTPA ester of squamosten-A
13. Physico-chemical properties of squamocin-N (**14**)
14. ¹H-NMR data of squamocin-N (*R*)- and (*S*)-MTPA ester
15. Physico-chemical properties of squamocin-E (**15**)
16. ¹H-NMR data of (*R*)- and (*S*)-MTPA esters of squamocin-H and (*R*)-MTPA ester of squamocin-E
17. Physico-chemical properties of squamocin-B (**16**)
18. Physico-chemical properties of squamostanal-A (**17**)
19. Cytotoxicity of tetrahydrofuranic acetogenins against L1210
20. Ionophore assay of tetrahydrofuranic acetogenins

References

- 1) Alali, F. Q., X. -X. Liu, and J. L. McLaughlin, (1999) : Annonaceous acetogenins: recent progress. *J. Nat. Prod.*, **62**, 504-540.
- 2) Alfonso, D., T. C. Saizarbitoria, Z. -X. Zhao, G. Shi, Q. Ye, J. T. Schwedler, and J. L. McLaughlin (1996) : Aromin and aromicin, two new bioactive annonaceous acetogenins, possessing an unusual bis-THF ring structure, from *Xylopia aromatica* (annonaceae) . *Tetrahedron*, **52**, 4215-4224.
- 3) Alkofahi, A., J. K. Rupprecht, D. L. Smith, C. J. Chang, and J. L. McLaughlin (1988) : Goniiothalamycin and annonacin: bioactive acetogenins from *Goniiothalamus giganteus* (annonaceae) . *Experientia*, **44**, 83-85.
- 4) Araya, H., N. Hara, Y. Fujimoto, A. Srivastava, and M. Sahai (1994a) : Squamosten-A, a novel mono-tetrahydrofuranic acetogenins with a double bond in the hydrocarbon chain, from *Annona squamosa* L.. *Chem. Pharm. Bull.*, **42**, 388-391.
- 5) Araya, H., N. Hara, Y. Fujimoto, and M. Sahai (1994b) : Squamostanal-A, apparently derived from tetrahydrofuran acetogenin, from *Annona squamosa*. *Biosci. Biotech. Biochem.*, **58**, 1146-1147.
- 6) Araya, H., Y. Fujimoto, and K. Hirayama, (1994c) : Structural elucidation of tetrahydrofuranic acetogenins by means of precursor-ion scanning method. *J. Syn. Org. Chem.*, **52**, 765-777.
- 7) Araya, H., Y. Ohmae, S. Hiradate, and Y. Fujii (2001) : Plant growth inhibitory activity of annonaceous plant leaves - bioactivity of annonaceous tetrahydrofuran acetogenins to lettuce seedlings. *Latinoamericana de Quimica*, **29**, 148.
- 8) Araya, H., M. Sahai, S. Singh, A. K. Singh, M. Yoshida, N. Hara, and Y. Fujimoto (2002) : Squamocins-O₁ and -O₂, new adjacent

- bis-tetrahydrofuranic acetogenins from the seeds of *Annona squamosa*. *Phytochemistry*, **61**, 999-1004.
- 9) Araya, H., U. Maeda, N. Hara, M. Sahai, and Y. Fujimoto (2004) Annonaceous tetrahydrofuran acetogenins from *Annona reticulata* Seeds. Preparation for manuscript.
 - 10) Aspinall, D., L. G. Paleg, and F. T. Addicott (1967) : Abscisin and some hormone-regulated plant responses. *Aust. J. Biol. Sci.*, **20**, 869-882.
 - 11) Born, L., F. Lieb, J. P. Lorentzen, H. Moeschler, M. Nonfon, R. Sollner, and D. Wendisch (1990) : The relative configuration of acetogenins isolated from *Annona squamosa*: annonin I (squamocin) and annonin VI. *Planta Med.*, **56**, 312-316.
 - 12) Cahn, R. S., S. C. Ingold, and V. Prelog (1966) : Specification of molecular chirality. *Angew. Chem., Int. Ed.*, **5**, 385-415.
 - 13) Carlsen, S. H. J., T. Katsuki, V. S. Martin, and K. B. Sharpless (1981) : Grately improved procedure for ruthenium tetroxide catalyzed oxidations for organic compounds. *J. Org. Chem.*, **46**, 3936-3938.
 - 14) Casiraghi, G., F. Zanardi, L. Battistini, G. Rassu, and G. Appendino (1998) : Current advances in the chemical synthesis of annonaceous acetogenins and relatives. *Chemtracts Org. Chem.*, **11**, 803-827.
 - 15) Chen, C. -Y., F. -R. Chang, H. -F. Chiu, M. -J. Wu, and Y. -C. Yu (1999) : Aromin-A, an annonaceous acetogenin from *Annona cherimola*. *Phytochemistry*, **51**, 429-433.
 - 16) Coner, E. J. C. (1969) : Zusetsu Nettai Syokubutu Syusei, p.106-118, Hirokawa Shoten, Tokyo.
 - 17) Cortes, D., S. H. Myint, A. Laurens, R. Hocquemiller, M. Leboeuf, and A. Cave (1991) : Corossolone and corossoline, two novel cytotoxic mono-tetrahydrofuran γ -Lactone. *Can. J. Chem.*, **69**, 8-11.
 - 18) Desipderio, D. M., (1991) : Mass spectrometry of peptides, CRC Press, Boston.
 - 19) Dodds, J. H., and L. W. Roberts (1982) : Initiation and maintenance of callus, p.36-49. *In* Experiments in plant tissue culture, Cambridge University Press, Cambridge.
 - 20) Fang, X. -P., R. Song, Z. -M. Gu, M. J. Rieser, L. R. Miesbauer, D. L. Smith, and J. L. McLaughlin (1993a) : A new type of cytotoxic acetogenin: giganin from *Goniothalamus giganteus*. *Bioorg. Med. Chem. Lett.*, **3**, 1153-1156.
 - 21) Fang, X. -P., M. J. Rieser, Z. -M. Gu, G. -X. Zhao, and J. L. McLaughlin (1993b) : Annonaceous acetogenins: an updated review. *Phytochem. Anal.*, **4**, 27-67.
 - 22) Figadere, B. (1995) : Syntheses of acetogenins of annonaceae: a new class of bioactive polyketides. *Acc. Chem. Res.*, **28**, 359-365.
 - 23) Fujimoto, Y., T. Eguchi, K. Kakinuma, N. Ikekawa, M. Sahai, and Y. K. Gupta, (1988) : Squamocin, a new cytotoxic bis-tetrahydrofuran containing acetogenin from *Annona squamosa*. *Chem. Pharm. Bull.*, **36**, 4802-4806.
 - 24) Fujimoto, Y., C. Murasaki, K. Kakinuma, T. Eguchi, N. Ikekawa, M. Furuya, K. Hirayama, T. Ikekawa, M. Sahai, Y. K. Gupta, and A. B. Ray (1990) : Squamostatin-A: unprecedented bis-tetrahydrofuran acetogenin from *Annona squamosa*. *Tetrahedron Lett.*, **31**, 535-538.
 - 25) Fujimoto, Y., C. Murasaki, H. Shimada, S. Nishioka, K. Kakinuma, S. Singh, M. Singh, Y. K. Gupta, and M. Sahai (1994) : Annonaceous acetogenins from the seeds of *Annona squamosa*. non-adjacent bis-tetrahydrofuranic acetogenins. *Chem. Pharm. Bull.*, **42**, 1163-1174.
 - 26) Gale, J. B., J. -G. Yu, X. -E. Hu, A. Khare, D. K. Ho, and J. M. Cassady (1993a) : Stereochemistry of mono-tetrahydrofuranyl moiety in cytotoxic polyketides. part A: synthesis of model compounds. *Tetrahedron Lett.*, **34**, 5847-5850.
 - 27) Gale, J. B., J. -G. Yu, A. Khare, X. -E. Hu, D. K. Ho, and J. M. Cassady (1993b) : Stereochemistry of mono-tetrahydrofuranyl moiety in cytotoxic polyketides. part B: application of proton chemical shift patterns. *Tetrahedron Lett.*, **34**, 5851-5854.
 - 28) Gleye, C., A. Laurens, R. Hocquemiller, N. Faucheur, L. Serani, and O. Laprevote (1998) : Mass spectrometry and structure elucidation of 15-palmitoyl- and 15-oleyl-solamin, the first known fatty acid esters of acetogenins from *Annona muricata* L.. *Rapid Commun. Mass Spectrom.*, **12**, 1051-1056.
 - 29) Gleye, C., A. Laurens, O. Laprevote, L. Serani, and R. Hocquemiller (1999) : Isolation and structure elucidation of sabadelin, an

- acetogenin from roots of *Annona muricata*. *Phytochemistry*, **52**, 1403-1408.
- 30) Greene, T. W., and P. G. M. Wuts (1999) : Protection for the hydroxyl group, including 1,2- and 1,3-diols: acetate ester, p.150-160. In *Protective groups in organic synthesis*, 3rd ed., John Wiley & Sons, Inc., New York.
- 31) Gromek, D., B. Figadere, R. Hocquemiller, and A. Cave (1993) : Corepoxytone, a possible precursor of mono-tetrahydrofuran γ -lactone acetogenins: biomimetic synthesis of corossolone. *Tetrahedron*, **49**, 5247-5252.
- 32) Gu, Z. -M., X. -P. Fang, L. Zeng, and J. L. McLaughlin (1994a) : Goniocin from *Goniiothalamus giganteus*: the first tri-THF acetogenin. *Tetrahedron Lett.*, **35**, 5367-5368.
- 33) Gu, Z. -M., L. Zeng, X. -P. Fang, T. C. Saizarbitoria, M. Huo, and J. L. McLaughlin, (1994b) : Determining absolute configurations of stereocenters in annonaceous acetogenins through formaldehyde acetal derivatives and Mosher ester methodology. *J. Org. Chem.*, **59**, 5162-5172.
- 34) Gu, Z. -M., G. -X. Zhao, N. H. Oberlies, L. Zeng, and J. L. McLaughlin (1995) : Annonaceous acetogenins: potent mitochondrial inhibitors with diverse applications, p. 249-230. In *Phytochemistry of medicinal plants*, Plenum Press, New York.
- 35) Gu, Z. -M., D. Zhou, N. J. Lewis, J. Wu, G. Shi, and J. L. McLaughlin (1997) : Isolation of new bioactive annonaceous acetogenins from *Rollinia mucosa* guided chromatography/mass spectrometry. *Bioorg. Med. Chem.*, **5**, 1911-1916.
- 36) Hayashi, Y., K. Furusato, and T. Nakamura (1985) : Illustrated trees in colour, p.104, Hokuryukan co., LTD. Tokyo.
- 37) Hoppe, R., and H. -D. Scharf (1995) : Annonaceous acetogenins - synthetic approaches towards a novel class of natural products. *Synthesis*, 1447-1464.
- 38) Hopwood, D. A., and D. H. Sherman, (1990) : Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. *Annu. Rev. Genet.*, **24**, 37-66.
- 39) Hotta, M., K. Ogata, A. Nitta, K. Hoshikawa, M. Yanagi, and K. Yamazaki (1989) : Useful plants of the world, Heibonsha LTD, Tokyo.
- 40) Hoye, T. R., and J. C. Suhadolnik (1987) : On the stereochemistry of the bistetrahydrofuranyl moiety of uvaricin: proton chemical shifts can play a crucial role in complex structure determination. *J. Am. Chem. Soc.*, **109**, 4402-4403.
- 41) Hoye, T. R., and Z. -P. Zhuang (1988) : Validation of the ¹H-NMR chemical shift method for determination of stereochemistry in the bis(tetrahydrofuranyl) moiety of uvaricin-related acetogenins from annonaceae: rolliniastatun 1 (and asimicin). *J. Org. Chem.*, **53**, 5578-5580.
- 42) Iwasa, S. (2001) : Tropical fruit trees, illustrated, JIRCAS International Agriculture Series No.11, p.56-82, JIRCAS, Tsukuba, Japan.
- 43) Jolad, S. D., J. J. Hoffmann, K. H. Schram, J. R. Cole, M. S. Tempesta, G. R. Kriek, and R. B. Bates (1982) : Uvaricin, a new antitumor agent from *Uvaria accuminata* (annonaceae). *J. Org. Chem.*, **47**, 3151-3153.
- 44) Kawazu, K., J. P. Alcantara, and A. Kobayashi (1989) : Isolation and structure of neoannonin, a novel insecticidal compound from the seeds of *Annona squamosa*. *Agric. Biol. Chem.*, **53**, 2719-2722.
- 45) Kitagawa, I., and S. Katayama, unpublished data.
- 46) Laprevote, O., F. Roblot, R. Hocquemiller, A. Cave, B. Charles, and J. -C. Tabet (1991) : Structural elucidation of five stereoisomeric acetogenins, uleicins A-E, by tandem mass spectrometry. *Phytochemistry*, **30**, 2721-2727.
- 47) Leboeuf, M., A. Cave, F. K. Bhaumik, B. Mukherjee, and R. Mukherjee (1982) : The phytochemistry of the annonaceae. *Phytochemistry*, **21**, 2783-2813.
- 48) Li, X. -H., Y. -H. Hui, J. K. Rupprecht, Y. -M. Liu, K. V. Wood, D. L. Smith, C. -J. Chang, and J. L. McLaughlin (1990) : Bullatacin, bullatacinone, and squamone, a new bioactive acetogenin, from the bark of *Annona squamosa*. *J. Nat. Prod.*, **53**, 81-86.
- 49) Lin, Y. -Y., M. Risk, S. M. Ray, D. V. Engen, J. Clardy, J. Golik, J. C. James, and K. Nakanishi (1981) : Isolation and structure of brevetoxin B from the "red tide" dinoflagellate *Ptychodiscus brevis* (*Gymnodinium breve*). *J. Am. Chem. Soc.*, **103**, 6773-6775.

- 50) Lios, J. L., D. Cortes, and S. Valverde (1989) : Acetogenins, aporphinoids, and azaanthraquinone from *Annona cherimolia* seeds. *Planta Med.*, **55**, 321-323
- 51) Londershausen, M., W. Leicht, F. Lieb, H. Moeschler, and H. Weiss (1991) : Molecular mode of action of annonins. *Pestic. Sci.*, **33**, 427-438.
- 52) Mann, J., R. S. Davidson, J. B. Hobbs, D. V. Banthorpe, and J. B. Harborne (1994) : Natural products chemistry: their chemistry and biological significance, Longman Scientific & Technical, Essex.
- 53) Martin, J. M., S. R. Madigosky, Z. -M. Gu, D. Zhou, J. Wu, and J. L. McLaughlin (1999) : Chemical diffense in the zabra swallow-tail butterfly, *Eurytides marcellus*, involving annonaceous acetogenins. *J. Nat. Prod.*, **62**, 2-4.
- 54) McCloud, T. G., D. L. Smith, C. -J. Chang, and J. M. Cassady (1987) : Annonacin, a novel, biologically active polyketide from *Annona densicoma*. *Experientia*, **43**, 947-949.
- 55) Morita, H., Y. Sato, and J. Kobayashi (1999) : Cyclosquamosins A - G, cyclic peptides from the seeds of *Annona squamosa*. *Tetrahedron*, **55**, 7509-7518.
- 56) Murashige, T., and F. Skoog (1962) : A reviced medium for rapid growth and bio assay with tabacco tissue cultures. *Physiol. Plant.*, **15**, 473-497.
- 57) Murata, M., A. M. Legrand, Y. Ishibashi, M. Fukui, and T. Yasumoto (1990) : Structures and configurations of ciguatoxin from the Moray Eel *Gymnothorax javanicus* and its likely precursor from the dinoflagellate *Gambierdiscus toxicus*. *J. Am. Chem. Soc.*, **112**, 4380-4386.
- 58) Murata, M., H. Naoki, T. Iwashita, S. Matsunaga, M. Sasaki, A. Yokoyama, and T. Yasumoto (1993) : Structure of maitotoxin. *J. Am. Chem. Soc.*, **115**, 2060-2062.
- 59) Naganuma, S., K. Sakai, K. Hasumi, and A. Endo (1992) : Acaterin, a novel inhibitor of acyl-CoA: cholesterol acyltransferase produced by *Pseudomonas* sp. A92. *J. Antibiotics*, **45**, 1216-1221.
- 60) Nair, S., P. K. Gupta, and A. F. Mascarenhas (1983) : Haploid plants from in vitro anther culture of *Annona squamosa* Linn. *Plant Cell Rep.*, **2**, 198-200.
- 61) Nair, S., P. K. Gupta, M. V. Shirgurkar, and A. F. Mascarenhas, (1984) : *In vitro* organogenesis from leaf explants of *Annona squamosa* Linn. *Plant Cell Tissue Organ Culture*, **3**, 29-40.
- 62) Nishioka, N. (1988) : Indo-Hanatsudzuri: Indo-Shokubutsu-shi, p.119-122. Mokusei-sha, Tokyo.
- 63) Nishioka, S., H. Araya, C. Murasaki, M. Sahai, and Y. Fujimoto (1994) : Determination of absolute chemistry at carbinol centers of tetrahydrofuranic acetogenins by advanced Mosher method. *Nat. Prod. Lett.*, **5**, 117-121.
- 64) Nitsch, C. and J. P. Nitsch (1967) : The induction of flowering *in vitro* in stem segments of *Plumbago indica* L. - the production of vegetative buds.. *Planta*, **72**, 355-370.
- 65) Nonfon, M., F. Lieb, H. Moeschler, and Wendisch (1990) : Four annonins from *Annona squamosa*. *Phytochemistry*, **29**, 1951-1954.
- 66) Ohtani, I., T. Kusumi, Y. Kashman, and H. Kakisawa (1991) : High-field FT NMR application of Mosher's method. the absolute configurations of marine terpenoids. *J. Am. Chem. Soc.*, **113**, 4092-4096.
- 67) Pettit, G. R., G. M. Cragg, J. Polonsky, D. L. Herald, A. Goswami, C. R. Smith, C. Moretti, J. M. Schmidt, and D. Weisleder (1987) : Isolation and structure of rolliniastatin 1 from the south american tree *Rollinia mucosa*. *Can. J. Chem.*, **65**, 1433-1435.
- 68) Peyrat, J. -F., J. Mahuteau, B. Figadere and A. Cave (1997) : NMR - studies of Ca²⁺ complexes of annonaceous acetogenins. *J. Org. Chem.*, **62**, 4811-4815.
- 69) Queiroz, E. F., F. Roblot, A. Cave, R. Hocquemiller, L. Sarani, O. Laprevote and M. D. Q. Paulo, (1999) : Bistetrahydrofuran acetogenin from the roots of *Annona salzmanii*. *J. Nat. Prod.*, **62**, 710-713.
- 70) Rasai, S., A. P. George, and A. S. Kantharajah (1995) : Tissue culture of *Annona* sp. (cherimoya, atemoya, sugar apple and sour-

- sop) : a review. *Scientia Horticulture*, **62**, 1-14.
- 71) Rieser, M. J., Y. -H. Hui, J. K. Rupprecht, J. F. Kozłowski, K. V. Wood, J. L. McLaughlin, P. R. Hanson Z. Zhuang, and T. R. Hoye (1992) : Determination of absolute configuration of stereogenic carbinol centers in annonaceous acetogenins by ¹H- and ¹⁹F-NMR analysis of Mosher ester derivatives. *J. Am. Chem. Soc.*, **114**, 10203-10213.
- 72) Rieser, M. J., X. -P. Fang, J. E. Anderson, L. R. Miesbauer, D. L. Smith, and J. L. McLaughlin (1994) : Muricatetrocins A and B and gigantetrocin B: three new cytotoxic monotetrahydrofuran-ring acetogenins from *Annona muricata*. *Helv. Chim. Acta*, **76**, 2433-2444; Erratum, *ibid*, **77**, 882.
- 73) Rupprecht, J. K., Y. -H. Hui, and J. L. McLaughlin (1990) : Annonaceous acetogenins: a review. *J. Nat. Prod.*, **53**, 237-278.
- 74) Sahai, M., S. Singh, M. Singh, Y. K. Gupta, S. Akashi, R. Yuji, K. Hirayama, H. Asaki, H. Araya, N. Hara, T. Eguchi, K. Kakinuma, and Y. Fujimoto (1994) : Annonaceous acetogenins from the seeds of *Annona squamosa*. adjacent bis-tetrahydrofuranic acetogenins. *Chem. Pharm. Bull.*, **42**, 1163-1174.
- 75) Saizarbitoria, T. C., Z. -M. Gu, G. -X. Zhao, L. Zeng, J. F. Kozłowski and J. L. McLaughlin (1995) : Venezenin: a new bioactive annonaceous acetogenin from the bark of *Xylopiia aromatica*. *J. Nat. Prod.*, **58**, 532-539.
- 76) Santos, L. P., M. A. D. Boaventura, N. -J. Sun, J. M. Cassady, and A. B. de Oliveira (1996) : Araticulin, a bis -tetrahydrofuran polyketide from *Annona crassiflora* seeds. *Phytochemistry*, **42**, 705-707.
- 77) Sasaki, S., K. Maruta, H. Naito, H. Sugihara, K. Hiratani, and M. Maeda (1995) : New calcium-selective electrodes based on annonaceous acetogenins and their analogs with neighboring bistetrahydrofuran. *Tetrahedron Lett.*, **36**, 5571-5574.
- 78) Sasaki, S., K. Maruta, H. Naito, R. Maemura, E. Kawahara, and M. Maeda (1998) : Novel acyclic ligands for metal cations based on the adjacent bistetrahydrofuran as analogs of natural annonaceous acetogenins. *Tetrahedron*, **54**, 2401-2410.
- 79) Saszarbitoria, T. C., H. A. Johnson, F. Q. Alali, D. C. Hopp, L. L. Rogers, and J. L. McLaughlin (1998) : Annojahnin from *Annona Jahniit* a possible precursor of mono-tetrahydrofuran acetogenins. *Phytochemistry*, **49**, 1609-1616.
- 80) Schmitz, F. J., K. W. Kraus, L. S. Ciereszko, D. H. Sifford, and A. J. Weinheimer, (1966) : Ancepsenolide: a novel bisbutenolide of marine origin. *Tetrahedron Lett.*, 94-104.
- 81) Sekiyama, Y., H. Araya, K. Hasumi, A. Endo, and Y. Fujimoto (1997) : Biosynthesis of acaterin: isolation of 4,5-dihydroacaterin and its conversion into acaterin. *Nat. Prod. Lett.*, **11**, 61-66.
- 82) Sekiyama, Y., H. Araya, K. Hasumi, A. Endo, and Y. Fujimoto (1998) : Biosynthesis of acaterin: incorporation of glycerol into the C₃ branched unit. *Tetrahedron Lett.*, **39**, 6233-6236.
- 83) Sekiyama, Y., Y. Fujimoto, K. Hasumi, and A. Endo (1999) : Biosynthesis of acaterin : metabolic fate of *sn*-3 hydrogens of glycerol during the formation of 4-dehydroacaterin. *Tetrahedron Lett.*, **40**, 4223-4226.
- 84) Sekiyama, Y., Y. Fujimoto, K. Hasumi, and A. Endo (2001) : Biosynthesis of acaterin: coupling of C₅ unit with octanoate. *J. Org. Chem.*, **66**, 5649-5654.
- 85) Shi, G., D. Alfonso, M. O. Fatope, L. Zeng, Z. -M. Gu, G. -X. Zhao, K. He, J. M. McDougal, and J. L. McLaughlin (1995) : Mucocin: a new annonaceous acetogenins bearing a tetrahydropyran ring. *J. Am. Chem. Soc.*, **117**, 10409-10410.
- 86) Shi, G., J. F. Kozłowski, J. T. Schwedler, K. V. Wood, J. M. McDougal, and J. L. McLaughlin (1996) : Muconin and mucoxin: additional nonclassical bioactive acetogenins from *Rollinia mucosa*. *J. Org. Chem.*, **61**, 7988-7989.
- 87) Shi, G., J. M. McDougal, and J. L. McLaughlin (1997a) : Bioactive annonaceous acetogenins from *Rollinia mucosa*. *Phytochemistry*, **45**, 719-723.
- 88) Shi, G., K. He, X. Liu, Q. Ye, J. M. McDougal, and J. L. McLaughlin, J. L. (1997b) : A novel application of Mosher's method to epimeric carbinols in acetogenins. absolute configurations of 12-hydroxybullatacins A and new acetogenins from *Rollinia mucosa*. *Nat. Prod. Lett.*, **10**, 125-132.
- 89) Shimada, H., S. Nishioka, S. Singh, M. Sahai, and Y. Fujimoto (1994) : Absolute stereochemistry of non-adjacent bis-tetrahydro-

furanic acetogenins. *Tetrahedron Lett.*, **35**, 3961-3964.

- 90) Shinada, T., K. Murata, K. Hayashi, Y. Ooyama, Y. Nakagawa, Y. Ohfuné, T. Fujita, M. Hisada, L. Dai, H. Noaki, and T. Nakajima (2001) : Structure analysis of nitrogen-containing natural products by MS/MS using squaric acid as a trigger of charge-remote fragmentation. *43rd Symposium on the Chemistry of Natural Products: Symposium Papers*, p.187-192, Osaka, Japan.
- 91) Tokyo Kasei Kogyo Co., LTD., Labeling reagent for HPLC, Sheet Mo. AZ-502.
- 92) Torssell, K. B. G. (1983) : Natural product chemistry: a mechanistic and biosynthetic approach to secondary metabolism, John Wiley & Sons Limited, New York.
- 93) Vath, J. E., M. Zollinger, K. Biemann, and Z. Fresenius (1988) : Method for the derivatization of organic compounds at sub-nanomol level with reagent vapor. *Anal. Chem.*, **331**, 248-252.
- 94) Weber, H., and H. G. Khorana (1972) : Total synthesis of the structural gene for an alanine transfer ribonucleic acid from yeast. chemical synthesis of an icosadeoxynucleotide corresponding to the nucleotide sequence 21 to 40. *J. Mol. Biol.*, **72**, 219-249.
- 95) Xu, L., and C. -J. Chang (1989) : Chemistry and selective cytotoxicity of annonacin-10-one, isoannonacin, and isoannonacin-10-one. novel polyketides from *Annona densicoma* (annonaceae) . *J. Org. Chem.*, **54**, 5418-5421.
- 96) Yamada, A., K. Yamaguchi, H. Kitamura, K. Yazawa, S. Fukuzawa, G. -Y. -S. Wang, M. Kuramoto, and D. Uemura (1995) : Chemical substances regulating biofilm formation. *37rd Symposium on the Chemistry of Natural Products: Symposium Papers*, p.224-229, Tokushima, Japan.
- 97) Yasumoto, T., T. Igarashi, A. -M. Legrand, P. Cruchet, M. Chinain, T. Fujita, and H. Naoki (2000) : Structural elucidation of ciguatoxin congeners by fast-atom bombardment tandem mass spectroscopy. *J. Am. Chem. Soc.*, **122**, 4988-4989.
- 98) Yoshida, M. et al., unpublished data.
- 99) Yoshida, M., W. Feng, K. Nishio, M. Takahashi, Y. Heike, N. Saijo, and H. Ikekawa, (2001) : Antitumor action of the PKC activator gnidimacrin through CDK2 inhibition. *Int. J. Cancer*; **94**, 348-352.
- 100) Zeng, L., Q. Ye, N. H. Oberlies, G. Shi, Z. -M. Gu, K. He, and J. L. McLaughlin (1996) : Recent advances in annonaceous acetogenins. *Nat. Prod. Rep.*, **13**, 275-306.

バンレイシ (*Annona squamosa* L.) 種子に含まれる バンレイシ科テトラヒドロフランアセトゲニン類の 構造決定、生理活性

荒 谷 博

摘 要

バンレイシ科植物 (annonaceae) は約 130 属 2300 種以上からなる大きな科である。この科の植物には古くから薬用植物として使用されてきたものが数多く存在する。しかし、これらの植物の主な生育分布が熱帯および亜熱帯地域であることもあり、その化学的組成研究はあまり進展しておらず、最近になりようやく本格的に研究の対象として使用されるようになった。近年、この科の *Annona*, *Asimina*, *Rollinia*, *Uvaria* などの限られた属 (Table 1) の植物種からテトラヒドロフランアセトゲニン類 (tetrahydrofuran acetogenins) あるいはバンレイシ科アセトゲニン類 (annonaceous acetogenins) と総称される化合物が単離・報告されるようになった。これらの化合物は強い抗腫瘍活性、細胞毒性、殺虫活性、免疫抑制活性などの顕著生理活性を有し、さらに広範な活性スペクトルを示すことから大きな注目を浴びている。

一般的に、テトラヒドロフランアセトゲニン類は脂肪鎖上に 1 ~ 3 個のテトラヒドロフラン環、2 ~ 7 個の水酸基を主とした酸素官能基、末端に 1 つの α,β -不飽和- γ -ラクトンを有する。例として本研究における主化合物である直結ビステトラヒドロフランアセトゲニン類に分類されるスクアモシン (squamocin) の構造を Fig. 6 に示した。この図から明らかなように、本化合物は直結した 2 つのテトラヒドロフラン環、3 つの水酸基、1 つの α,β -不飽和- γ -ラクトン部に 8 つの不斉炭素を有する脂肪酸誘導体である。

著者はトロピカルフルーツとして知られる *Annona squamosa* L. (英名: sugar apple、和名: バンレイシ、釈迦頭) 種子中から、7 種の新規テトラヒドロフランアセトゲニン類を単離・構造決定し、16 種の既知アセトゲニン類を単離・同定した。また、これら化合物の平面構造を一義的に決定する新規手法 (アミン法) を開発した。本論文 (英文) は以下の 5 章より構成される。

第 1 章 緒言

第 2 章 テトラヒドロフランアセトゲニン類の平面構造決定のためのプリカーサーイオンスキャン法の適用

第 3 章 新規テトラヒドロフランアセトゲニン類の構造決定

第 4 章 結果および考察

第 5 章 実験の部

第 1 章 (緒言: Introduction) ではバンレイシ科植物における天然有機化合物研究の現状、特にテトラヒドロフランアセトゲニン類を中心にレビューした。また、テトラヒドロフランアセトゲニン類の構造決定研究における現状、困難点および問題点を指摘した。

第 2 章 (テトラヒドロフランアセトゲニン類の平面構造決定のためのプリカーサーイオンスキャン法の適用: Application of Precursor Ion Scanning Method for Planar Structure Elucidation of Tetrahydrofuran Acetogenins) では、新規に開発した *N,N*-ジメチルエチレンジアミンによる誘導体化とプリカーサーイオンスキャン法を組み合わせた手法 (アミン法) の確立の過程について述べた。

本法はテトラヒドロフランアセトゲニン類の平面構造を一義的に決定できる画期的な方法である。テトラヒドロフランアセトゲニン類は先に図示したスクアモシンの構造からも想像されるように、その平面構造はNMRのみの解析では決定できず、MSスペクトルの解析が必要不可欠である。一般的によく利用されているEIおよびCI-MSスペクトルではグリコール部分の開裂を示すフラグメントイオンは十分な強度で観測されるが、脂肪鎖上の水酸基などの酸素官能基の位置を示すフラグメントイオン強度が低く、NMRスペクトル解析と併せた慎重な解析が必要となる。実際に、複数のグループが間違った平面構造を報告している。

本アミン法をスクアモシンに適用した結果をFig. 27に例示した。

この図から明らかなように、ほぼすべての炭素-炭素結合の開裂を示すフラグメントイオンがはっきりと観測されるため、スクアモシンの平面構造は一義的に決定できる。

本論文では、スクアモシンの他に直結ビステトラヒドロフラン型のスクアモシン-C、スクアモシン-F、2つのテトラヒドロフラン環が4炭素隔てられて結合した非直結ビステトラヒドロフラン型のスクアモスタチン-C、さらに脂肪鎖上にカルボキシル基を有するモノテトラヒドロフラン型のアノナシン-10-オンに本法を適用した結果について記述した。

第3章（新規テトラヒドロフランアセトゲニン類の構造決定：Structure Elucidation of New Tetrahydrofuran Acetogenins）では単離した7種のテトラヒドロフランアセトゲニン類（スクアモシン-O₁、-O₂、-N、-E、-B、スクアモステン-Aおよびスクアモスタナル-A）の単離・構造決定について述べた。これらの構造決定は必要に応じて、第2章で述べたアミン法を適用した。

テトラヒドロフランアセトゲニン類はオイル状もしくは微結晶の形状のためX線結晶解析が困難であるため、これらの絶対立体配置は新Mosher法や円二色性スペクトルなどの解析により、すべての新規アセトゲニンについて全不斉炭素の絶対立体配置を決定した。

新規化合物7種は以下の構造的特徴を有していた。

squamocins-O₁、-O₂：C-12位の立体がエピメリックな関係であるビステトラヒドロフランアセトゲニンの対である世界で2番目の例

squamocins-B、-E：単離報告の少ない炭素数が35のビステトラヒドロフランアセトゲニン

squamocin-N：テトラヒドロフラン周辺部の相対配置が *threo/cis/threo/cis/trans* である世界で唯一の化合物

squamosten-A：二重結合を有するモノテトラヒドロフランアセトゲニンである。生合成的に興味深い化合物

squamostanal-A：ラクトン環を含むA鎖部分のみで構成される世界で唯一の化合物

第4章（結果および考察：Results and Discussion）では1）新規開発したアミン法の誘導体生成メカニズム、適用時の留意点、2）単離・構造決定したテトラヒドロフランアセトゲニン類の構造、3）テトラヒドロフランアセトゲニン類の生合成仮説、4）テトラヒドロフランアセトゲニン類の生理活性（細胞毒性、イオノフォア活性およびレタス発芽種子生長阻害活性）、5）テトラヒドロフランアセトゲニン類の研究の今後の課題、について結果を示し、考察を行った。

第5章（実験の部；Experimental Section）では、誘導体化、分解反応を含めた各種反応条件の詳細、単離条件について詳述した。さらに、本研究において単離・構造決定した新規7種、既知16種、計23種のテトラヒドロフランアセトゲニン類の物理化学的性質および各種スペクトルデータを記載した。