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

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Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat
Studies on Annonaceous Tetrahydrofuranic A cetogenins 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.

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Chapter 1
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; Torsell, 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 (Leboeuf et al., 1982; Morita et al., 1999); Fig. 2). However, a series of compounds termed tetrahydrofuranic acetogenins (or
annoaceous 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.

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 \( \alpha, \beta \)-unsaturated-\( \gamma \)-lactone at its 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).

<table>
<thead>
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<th>Table 1. Genera of source of tetrahydrofuranic acetogenins</th>
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<tr>
<td><strong>Annona</strong></td>
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<tr>
<td><em>Annona muricata</em></td>
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<tr>
<td><em>Asimina</em></td>
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<td><em>Disepatium</em></td>
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<td><em>Goniothalmus</em></td>
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The fundamental structural features of tetrahydrofuranic acetogenins are:

1. Having hydrocarbon chain, of C35 or C37 in length.
2. 1\( \rightarrow \) 3 tetrahydrofuran rings are present.
3. One \( \gamma \)-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.

![Type A](image1)

Type A

![Type B](image2)

Type B

![Type C](image3)

Type C

![Type D](image4)

Type D

![Fig. 4. Classification of tetrahydrofuran portion](image5)

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) \(\alpha,\beta\)-unsaturated-\(\gamma\)-lactone
(b) ketolactone type
(c) \(\gamma\)-hydroxy lactone
(d) \(\beta\)-hydroxy lactone

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

![Type (a)](image6)

Type (a)

![Type (c)](image7)

Type (c)

![Type (b)](image8)

Type (b)

![Type (d)](image9)

Type (d)

![Fig. 5. Structures of lactone moiety](image10)

Type (a')

Type (c)
(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 microcrystal 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 below 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 1H-NMR data between per-acetylated acetogenin.
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 $\alpha$-methoxy-$\alpha$-(trifluoromethyl)phenylacetyl (MTPA) esters (as described in Chapter ⅴ).

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

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<td>6</td>
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* The left column indicates 6:5:2:2′:2′: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 hydroxyl groups 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

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<tr>
<td>OH</td>
<td>3.84/71.83</td>
<td>3.84/82.29</td>
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<tr>
<td>OH</td>
<td>3.40/73.87</td>
<td>3.79/82.47</td>
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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](image)

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 $^1$H- 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.

![goniocin](image)

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).

![giganin](image)

![venezenin](image)

![sabadelin](image)

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  altrovert). 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  altrovert). In Chapter  altrovert, 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  altrovert.
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, acetoxyl, 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.
Derivatization to acetate, trimethylsilyl (TMS) ether cannot 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 $\alpha$-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.

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.
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 - H₂O), 501 (C-28/C-29 - 2H₂O)) 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⁺ - H₂O), 587 (MH⁺ - 2H₂O), 569 (MH⁺ - 3H₂O), 551 (MH⁺ - 4H₂O), 533 (MH⁺ - 5H₂O)) were seen.

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

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), 519 (C-28/C-29 - H₂O), 501 (C-28/C-29 - 2H₂O) which indicate an existence of 28-OH on b-chain, were not detected clearly.

On the other hand, ions m/z 327 (C-15/C-16), 309 (C-15/C-16 - H₂O), 239 (C-19/C-20 - H₂O) 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.
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.

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.
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 (CHCl₃/AcOEt/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 C₅₁H₈₂O₆N₂ 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 ¹³C-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

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*This Table lists the data around lactam.*

---

Table 4. NMR data of benzylamine derivative (4)

![Fig. 17. Predictable reaction of α,β-unsaturated-γ-lactone with amines](image17)

![Fig. 18. Structure of benzylamine derivative (4)](image18)
groups. $^{13}$C-NMR spectra analysis further showed a new N,N-acetal (aminal) carbon signal ca. $\delta$ 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](image1)

![Fig. 20. Precursor ion scanning from m/z 91 for the benzylamine (4)](image2)
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-dimethylethlenediamine

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-dimethylethlenediamine (Fig. 21). This reaction was performed in a neat condition: 2 (10 mg), N,N-dimethylethlenediamine (5 µl, 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.

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.

The derivatization was also tried in dioxane. Squamocin (2) 10 mg, N,N-dimethylethylenediamine 0.5 μl, dioxane 0.5 ml were sealed in a microtube, and heated at 80 °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 aminal 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)

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*1H-, 13C-NMR data not listed are very close to starting material (2).
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_{6}N_{4}, respectively. The IR (\nu_{\text{max}} 1670 cm\textsuperscript{-1}) and \textsuperscript{1}H-, \textsuperscript{13}C-NMR (Table 6) made clear that 6a and 6b had a \gamma-lactam and no double bond as in 5. In \textsuperscript{13}C-NMR spectra, an N,O-acetal carbon signals (\delta_c 85.93, 86.92) such as in derivative (5) disappeared, and a new N,N-acetal (aminal) carbon signals (\delta_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 \textsuperscript{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)

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\[^1\text{H}, \text{\textsuperscript{13}C}-\text{NMR data not listed are very close to starting material (2).}]}
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.

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 (Sahai et al., 1994), an adjacent bis-THF type acetogenin, has a hydroxyl group at C-29 on the b-chain. In the El-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 - H2O, 1.11 %), 497 (C-29/C-30 - 2H2O, 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 - H2O), 609 (C-28/C-29) and 591 (C-28/C-29 - H2O)] were clearly observed as sufficiently intense signals.
(2) Application of the amine method to squamocin-F (8)

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 - H₂O) and 293 (C-15/C-16 - H₂O) 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 - H₂O), 293 (C-11/C-12 - H₂O) clearly indicated the presence of hydroxyl group at C-12.
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).

(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. 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 a-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.
1. The isolation procedure

In this study, seven new acetogenins; squamocin-O\(^1\) \((11)\), -O\(^2\) \((12)\) (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.
Araya: Studies on Tetrahydrofuranic Acetogenins

Fig. 35. Isolated tetrahydrofuranic acetogenins in this study

Fig. 36. Isolation of new tetrahydrofuranic acetogenins

Ground *Anona squamosa* Seeds (2 kg)

1) Pet. Ether
2) Partition Between Pet. Ether and Aq. Methanol

Methanol Layer

Pet. Ether Layer

AcOEt

Residue

Crude Acetogenin Fraction

Silica gel Chromatog.

CHCl₃/MeOH = 2:1
to

AcOEt/MeOH = 2:1

Less Polar

Fr. 1 2 3 4 5 6 7 8 9 10

More Polar

Squamocin-N

Squamostanol-A

Squamarsten-A

Squaromicin-B

Squaromicin-O₂

Squamocin-O₂
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 CHCl₃/MeOEt and AcOEt/MEOH as eluting solvents. Ten fractions obtained were re-chromatographed on reversed phase HPLC (MeOH/H₂O or CH₃CN/H₂O as elution solvent). Accordingly, seven novel acetogenins; squamocin-O₁ (11) (14 mg), squamocin-O₂ (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-O₁ (11), -O₂ (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-O₁ (11) (more mobile isomer under the HPLC condition) and squamocin-O₂ (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 ($\lambda_{\text{max}}$ 210 nm) and IR ($\nu_{\text{max}}$ 1750 cm⁻¹) absorption typical of $\alpha,\beta$-unsaturated-$\gamma$-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 C₃₇H₆₆O₈, was deduced from HR-FAB-MS data (found, 639.4792 [(M+H)⁺] and 639.4835). The 1H- and 13C-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 ($\delta$H 3.8, $\delta$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](image)

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).
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 13C-NMR (Table 7) data on the basis of accumulated 13C-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.** 13C-NMR spectral data for squamocins-O 1 (11) and -O 2 (12)

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<td>25.1</td>
<td>27.3</td>
<td>82.8</td>
<td>82.9</td>
<td>71.3</td>
<td>71.2</td>
<td>32.4</td>
<td>32.5</td>
<td>22.1</td>
<td>22.2</td>
<td>37.4</td>
<td>37.4</td>
<td>37.4</td>
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<tr>
<td></td>
<td>26.9</td>
<td>4.6</td>
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<tr>
<td></td>
<td>83.1</td>
<td>83.2</td>
<td>28.4</td>
<td>28.4</td>
<td>28.9</td>
<td>29.0</td>
<td>82.2b</td>
<td>82.2c</td>
<td>82.5b</td>
<td>82.5c</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>28.9</td>
<td>29.0</td>
<td>35</td>
<td>145.9</td>
<td>19.2</td>
<td>19.2</td>
<td>77.4</td>
<td>77.4</td>
<td>77.4</td>
<td>6.5</td>
<td>4.5</td>
<td>24.6</td>
<td>24.6</td>
<td>24.6</td>
<td>24.6</td>
<td>24.6</td>
<td>24.6</td>
</tr>
</tbody>
</table>

a) The signals appeared in the region of ±25.0-30.0.

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 $^1$H-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. $^1$H-NMR spectral data for MTPA esters (11rs, 12rs and 2rs) |
|-----------------------|----------------|----------------|
| C | 11r | 12r | 2r |
| 4 | 2.265 (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) | 3.99 (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 | 0.880 (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 $D 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 $D(11r-12r) | - | +0.03 | +0.07 | +0.01 | +0.02 |
| $\Delta $D(18r-19r) | - | +0.02 | +0.07 | +0.01 | +0.01 |

R$_1$=O-(R)-MTPA, R$_2$=H: 18r
R$_1$=H, R$_2$=O-(R)-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, \(15R,16R,19R,20R,23R,24S\) and \(15S,16S,19S,20S,23S,24R\) 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 \(15R,16R,19R,20R,23R,24S\). 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 \(\Delta \delta (2r-3r)\) was identical to that of \(\Delta \delta (2r-3r)\), C-12 of \(11\) and \(12\) were concluded to have the respective R and S configurations, respectively. This result was in agreement of the \(^{13}C\)-NMR chemical shifts at C-12 and C-15 (δC \(11\): 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)](image)

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

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.W.</td>
<td>622 (FAB-MS: MH+ 623)</td>
</tr>
<tr>
<td>M.F.</td>
<td>(C_{27}H_{36}O_5) (HR-FAB-MS: 623.4844 (MH(^+), Calcd. 623.4887))</td>
</tr>
<tr>
<td>([\alpha]_D^{25})</td>
<td>+9.0 (c=0.10, MeOH)</td>
</tr>
<tr>
<td>UV ((\lambda_{max}) (\varepsilon))</td>
<td>210 nm (7000)</td>
</tr>
<tr>
<td>IR ((v_{max}))</td>
<td>3665, 3575, 3450, 1745 (cm(^{-1}), CHCl(_3))</td>
</tr>
<tr>
<td>CD (\Delta\varepsilon)</td>
<td>-0.57 (MeOH, at 240 nm)</td>
</tr>
</tbody>
</table>
methylene protons (\(\delta 2.53, 2.40\)) at C3 are coupled in an ABX type pattern, and \(^{13}\)C-NMR chemical shift at C-3, -4 and -5 (\(\delta 33.38, 69.99, 37.39\)) 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 (oxymethyne protons; \(^1\)H-NMR: \(\delta 3.44\) (2H), 3.83 (2H), and carbons; \(^{13}\)C-NMR: \(\delta 73.50, 74.37, 82.63, 83.65\)) indicated that 13 has (C1) type core. This fact was supported by Born's rule (Table 3) and an indication of existence of two -C-CH\(_2\)OMTPA-CH\(_2\)OR-C- structures (H\(_a\) and H\(_b\) are coupled) in \(^1\)H-NMR of its (R)-MTPA ester. The NMR data indicate the presence of two more secondary hydroxyl groups (\(\delta_{\text{H}} 3.63, \delta_{\text{C}} 71.70\)) and a double bond (\(\delta_{\text{H}} 5.36, 5.39, \delta_{\text{C}} 128.92, 130.87\)).

In the EI-MS spectra, fragment ions, m/z 309 (C-15/C-16 - H\(_2\)O), 379 (C-19/C-20 - H\(_2\)O) 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\(_2\)O) 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.

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 (\(\delta_{\text{C}} \approx 29\))). 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\(_2\)O), 309 (C-11/C-12 - H\(_2\)O) were observed clearly.

In order to determine the position of the double bond on the b-chain, compound 13 (55 \(\mu\)g) was treated with ruthenium oxidation (ruthenium trichloride, sodium periodate) (Carlsen et al., 1981), and the resulting fatty acid was
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 (JH-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, 13C-NMR with model compounds (the result of 13C-NMR was shown in Table 11) (Fujimoto et al., 1994).

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

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

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

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

Fig. 42. HPLC analysis of fatty acid p-bromophenacyl ester
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>
<thead>
<tr>
<th>Table 12. 1H-NMR data of (R)- and (S)-MTPA ester of squamocin-G and (R)-MTPA ester of squamosten-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R) -ester of Sq-G</td>
</tr>
<tr>
<td>(S) -ester of Sq-G</td>
</tr>
<tr>
<td>Δδ_{(1S,3S)}</td>
</tr>
<tr>
<td>(R)-ester of (13)</td>
</tr>
</tbody>
</table>

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 36S 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 12R,15R,16R,19R,20R.

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

(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 (α) type lactone lacking 4-OH, because methylene protons were observed at δ 2.26 as triplet (J = 7.7 Hz) in 1H-NMR.
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).

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

<table>
<thead>
<tr>
<th>State</th>
<th>White wax</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.W.</td>
<td>606 (FAB-MS: MH⁺ 607)</td>
</tr>
<tr>
<td>M.F.</td>
<td>C₁₉H₂₀O₈ (Anal. C, 73.01; H, 11.25 (Calcd. C, 73.22; H, 10.96))</td>
</tr>
<tr>
<td>[α]D25</td>
<td>+40.6 (c=0.43, MeOH)</td>
</tr>
<tr>
<td>UV (λmax, ε)</td>
<td>210 nm (7000)</td>
</tr>
<tr>
<td>IR (νmax, cm⁻¹)</td>
<td>3560, 3450, 1750 (MeOH, CHCl₃)</td>
</tr>
<tr>
<td>CD</td>
<td>Ac -0.57 (MeOH, at 240 nm)</td>
</tr>
</tbody>
</table>

The relative stereochemistry of the bis-tetrahydrofurans portion was determined to be threo/cis/threo/cis/threo by the Hoye'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 Hoye'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 15R,16R,19S,20S,23R,24R 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 desielding 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

<table>
<thead>
<tr>
<th></th>
<th>H₁₅−14, -25Δ</th>
<th>H-16, -23</th>
<th>H-19, 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R) ester</td>
<td>1.49-1.54</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>(S) ester</td>
<td>1.63-1.66</td>
<td>3.95</td>
<td>3.76</td>
</tr>
<tr>
<td>Δδ(15R)−(15S)</td>
<td>0.13</td>
<td>0.05</td>
<td>-0.14</td>
</tr>
<tr>
<td>presumed sign</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 15R,16R,19S,20S,23R,24R,36S absolute configuration.
(4) Structure of squamocin-E (15)

Physico-chemical property of squamocin-E (15) is shown in Table 15. The $^1$H- and $^{13}$C-NMR revealed that 15 has a (α') 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)

<table>
<thead>
<tr>
<th>State</th>
<th>White solid, mp. 48-50 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.W.</td>
<td>594 (FAB-MS: MH$^+$ 595)</td>
</tr>
<tr>
<td>M.F.</td>
<td>$\text{C}<em>{25}\text{H}</em>{40}\text{O}_{6}$ (HR FAB-MS: 595.4592 (MH$^+$, Calcd. 545.4574))</td>
</tr>
<tr>
<td>$[\alpha]_D^{25}$</td>
<td>+20.9 (c=0.25, MeOH)</td>
</tr>
<tr>
<td>UV ($\lambda_{max}$, loge)</td>
<td>209 nm (3.7)</td>
</tr>
<tr>
<td>IR ($\nu_{max}$)</td>
<td>3665, 3570, 3450, 1745 (cm$^{-1}$, CHCl$_3$)</td>
</tr>
<tr>
<td>CD</td>
<td>Ac -0.40 (MeOH, at 240 nm)</td>
</tr>
</tbody>
</table>

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 (M$^+$ - H$_2$O), 558 (M$^+$ - 2H$_2$O), 540 (M$^+$ - 3H$_2$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 13R,14R,17R,18R,21R,22R 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 4R,13R,14R,17R,18R,21R,22R,34S configuration as shown bellow having 35 carbons.
This compound \( \textbf{15} \) was a rare (A) type acetogenin having a carbon number 35. The NMR spectra \( ^1\text{H} \) and \( ^{13}\text{C} \) could not be distinguished with squamocin-H (=asimicin, molecular weight: 622, molecular formula: \( \text{C}_{37}\text{H}_{66}\text{O}_{7} \)).

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

<table>
<thead>
<tr>
<th></th>
<th>( \text{H}-14, -25^\text{a} )</th>
<th>( \text{H}-16, -23 )</th>
<th>( \text{H}-19, -20 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R) - ester of Sq-H</td>
<td>1.45</td>
<td>3.97</td>
<td>3.92</td>
</tr>
<tr>
<td>(S) - ester of Sq-H</td>
<td>1.56</td>
<td>3.93</td>
<td>3.76</td>
</tr>
<tr>
<td>( \Delta\delta_{(R,S)} )</td>
<td>0.11</td>
<td>-0.04</td>
<td>-0.16</td>
</tr>
<tr>
<td>(R) - ester of Sq-E</td>
<td>1.46</td>
<td>4</td>
<td>3.93</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>State</td>
<td>White wax</td>
</tr>
<tr>
<td>M.W.</td>
<td>594 (FAB-MS: MH(^+) 595)</td>
</tr>
<tr>
<td>M.F.</td>
<td>( \text{C}<em>{36}\text{H}</em>{65}\text{O}_{7} ) (HR-FAB-MS: 595.4592 (MH(^+), Calcd. 545.4574))</td>
</tr>
<tr>
<td>( [\alpha]_D^{25} )</td>
<td>+27.6 (c=0.25, MeOH)</td>
</tr>
<tr>
<td>UV (( \lambda_{max} ) loge)</td>
<td>209 nm (3.7)</td>
</tr>
<tr>
<td>IR (( \nu_{max} ))</td>
<td>3660, 3575, 3450, 1745 (cm(^{-1}), CHCl(_3))</td>
</tr>
<tr>
<td>CD</td>
<td>( \Delta\varepsilon -0.42 ) (MeOH, at 240 nm)</td>
</tr>
</tbody>
</table>

From the EI-MS spectra fragment pattern (Fig. 46), it was clear that a tetrahydropuran 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 \( ^{13}\text{C}-\text{NMR signal} \( \delta 21.99 \) (C-24).

**Fig. 46. EI-MS fragmentation pattern of squamocin-B (16)**
The $^1$H- and $^{13}$C-NMR data of this compound (16) were very close to that of squamocin (2). Further, $^1$H-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.

---

(6) Structure of squamostanal-A (17)

As shown in Table 18, molecular weight of this compound (17) was 294, and the molecular formula was C$_{19}$H$_{30}$O$_3$. An existence of $\alpha,\beta$-unsaturated-$\gamma$-lactone of (I) type was confirmed by UV, $^1$H- and $^{13}$C-NMR. The oxymethyne signals of NMR, observed in general tetrahydrofuranic acetogenins conventionally, was not observed, but a new aldehyde signal ($\delta$H 9.77 (t, $J$ = 1.9 Hz, H-15), $\delta$C 203.03 (C-15)) was observed. Since this aldehyde proton signal was coupled with methylene protons of $\delta$H 2.42, and signals $\delta$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)

<table>
<thead>
<tr>
<th>State</th>
<th>M.W.</th>
<th>M.F.</th>
<th>UV ($\lambda_{max}$, $\varepsilon$)</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>oil</td>
<td>294</td>
<td>C$<em>{19}$H$</em>{30}$O$_3$ (HR-FAB-MS: 295.2225 (MH, Calcd. 295.2273))</td>
<td>$\Lambda_\alpha$ -0.42 (MeOH, at 240 nm)</td>
<td></td>
</tr>
</tbody>
</table>
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 tetra-acetate oxidation of neoannonin (= squamocin-J) (Kawazu et al., 1989). 1H-NMR of the compound was very close to that of 17.
Chapter 1. Results and Discussion

1. The amine method

The author has established a new method (the amine method was described in Chapter 2), 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; Y asumoto 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
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.

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

In this study, seven new tetrahydrofuranic acetogens, 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, squamo-
statins-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); α-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).
Since most of tetrahydrofuranic acetogenins, isolated from A. squamosa L. seeds in this study, have 15R configuration (13R in C35 acetogenins), the biosynthetic pathway of the tetrahydrofuranic portion can be described as in Fig. 51.

![Proposed biosynthetic pathway of tetrahydrofuran moiety Annona squamosa L. seeds](image)

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.
4. Bioactivities of tetrahydrofuranic acetogenins

Among the wide spectral bioactivities of tetrahydrofuranic acetogenins pharmaceutical activities are most interesting. Twentythree 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type</th>
<th>ED₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sq-C</td>
<td>(A), C37, th/tr/th/tr/er</td>
<td>1.0 x 10⁻³</td>
</tr>
<tr>
<td>Sq-G</td>
<td>(A), C37, th/tr/th/tr/er</td>
<td>1.0 x 10⁻⁴</td>
</tr>
<tr>
<td>Sq-I</td>
<td>(A), C37, th/tr/th/tr/th</td>
<td>1.3 x 10⁻⁴</td>
</tr>
<tr>
<td>Sq-J</td>
<td>(A), C35, er/tr/th/tr/th</td>
<td>2.1 x 10⁻³</td>
</tr>
<tr>
<td>Sq-L</td>
<td>(A), C37, th/tr/tr/th/tr/er</td>
<td>2.0 x 10⁻⁴</td>
</tr>
<tr>
<td>St-D</td>
<td>(B), C37, tr/tri-th/tr/tr/er</td>
<td>2.2 x 10⁻⁴</td>
</tr>
<tr>
<td>St-E</td>
<td>(B), C37, tr/tri-th/tr/th</td>
<td>3.8 x 10⁻⁴</td>
</tr>
</tbody>
</table>

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 the 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).

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.
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 Ⅲ
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 13C at 125 MHz), JEOL EX-400 (1H at 400 MHz and 13C at 100 MHz), JEOL GSX-270 (1H at 270 MHz and 13C at 67.5 MHz) or Varian INOVA-400 (1H at 400 MHz and 13C at 100 MHz) spectrometer with CDCl3 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 ° in MeOH solution. IR spectra were determined on a JASCO IR-810 spectrometer in CHCl3 solution. Optical Rotations were measured with a JASCO DIP-360 polarimeter at 25 ° in MeOH solution. EI- (70 eV) and FAB-MS were obtained with a JEOL JMS-A X505HA 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)
A 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 ° 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 νmax (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 μl) in a micro tube, heating on an oil bath at 80 ºC 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 Rf 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 , sited in Chapter 2). Rf 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 νmax (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 μl) in a micro tube, heating on a 80 ºC 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 %).

(iii) Derivatization in dioxane

Squamocin (2) 10 mg, N,N-dimethylethylenediamine 0.5 ml were dissolved in dioxane 0.5 ml, and heated in an oil bath at 80 ºC 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-dimethylformamide 20 μg in a micro tube, and heated at 80 ºC for four hours. The reactant was dissolved in a small amount of ethyl acetate, followed by transferation 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 C₄₅H₇₉O₇N₂, 781.6782), IR νmax (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 below.

State: white needles, MP: 48.5-49 °C, $\left[\alpha\right]_D^{25} +15.0$ (c=1.7, MeOH), UV $\lambda_{\text{max}}$ (MeOH) nm (log e): 215 (3.5), IR $\nu_{\text{max}}$ (CHCl$_3$) cm$^{-1}$: 3680, 3585, 3460, 3015, 2940, 2855, 1755, $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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). $^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta$: 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)

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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)

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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 (IV) chloride and following p-bromophenacyl esterification of the fatty acid**

Squamosten-A (12) (55 mg), sodium periodate (100 mg), ruthenium (IV) chloride-n-H$_2$O (catalytic amount) were dissolved in H$_2$O-CH$_3$CN-CCl$_4$ solvent mixture (10 ml each) and stirred an hour (Carlsen et al., 1981). The reaction mixture was partitioned between aqueous NaHCO$_3$ and CCl$_4$ 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 mg), acetonitrile (100 ml), N,N-diisopropylethylamine (5 mg) 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: CH$_3$CN/H$_2$O (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), [α]D₂₅: +17.7 (c=0.6, MeOH), UV λmax (MeOH) nm (log ε): 210 (3.8), CD (MeOH) Δε (nm) -0.45 (239). IR νmax (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=6.4 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 M Hz, CDCl₃): Table 8.

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

¹H-NMR (500 M Hz, 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), [α]D₂₅: +17.4 (c=1.0, MeOH), UV λmax (MeOH) nm (log ε): 210 (3.8), CD (MeOH) Δε (nm) -0.45 (239). IR νmax (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=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-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 °C, [α]D₂₅: +9.0 (c = 0.10, MeOH), CD (MeOH) De₅₄ (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, d, 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-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), 30-39 (C-7, -8, -9, -14, -26, -27, -28, -29, -30, -31), 31.9 (C-32), 33.4 (C-3, -21), 37.4 (C-5), 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-16 or H-19), 4.99 (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, [α]D₂₅: +40.6 (c = 0.43, MeOH), CD (MeOH) De₅₄ (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-35), 6.98 (1H, d, J = 1.3 Hz, H-35), 13C-NMR (125 MHz, CDCl₃) δ: 14.1 (C-34), 19.1 (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 (2H, 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

$^1$H-NMR (500 M Hz, CDCl$_3$): $\delta$: 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, quii, 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 : H$_2$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 (CH$_3$CN : H$_2$O= 20 : 1, flow rate: 7.0 ml/min, retention time 21.0 min fraction was collected). Further purification by HPLC (MeOH : H$_2$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: C$_{35}$H$_{62}$O$_7$, State: white solid, MP: 77-78°C, $[\alpha]_D^{25}$: +20.9 (c=0.25, MeOH), CD (MeOH) De(nm): -0.40 (240), HR-FAB-MS: 595.4592 (Calcd for C$_{35}$H$_{63}$O$_7$ 595.4574), $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$: 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), 13C-NMR (125 MHz, CDCl$_3$): 14.1 (C-32), 19.1 (C-35), 22.6 (C-15, -20), 28.3 (C-13, -22), 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

$^1$H-NMR (500 MHz, CDCl$_3$): $\delta$: 0.88 (3H, t, J=5.6 Hz, H-32), 1.40 (3H, d, J=6.9 Hz, H-35), 2.54 (2H, m, H-3), 2.025 (3H, s, AcO), 2.076 (6H, s, 2 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), 7.08 (1H, br s, H-33).

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

$^1$H-NMR (500 M Hz, CDCl$_3$): $\delta$: 0.88 (3H, t, J=7.0 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 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), 7.08 (1H, br s, H-33).

Squamocin-B (16)

Ninth fraction shown Fig. 36 was chromatographed with HPLC (MeOH : H$_2$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 (CH$_3$CN : H$_2$O = 10 : 1, flow rate: 0.6 ml/min) afforded squamocin-B (16) (37 mg), MW: 594, MF: C$_{35}$H$_{62}$O$_7$, State: white wax, $[\alpha]_D^{25}$: +27.6 (c=0.25, MeOH), CD (MeOH) De(nm): -0.42 (240), HR-FAB-MS: 595.4630 (Calcd for C$_{35}$H$_{63}$O$_7$ 595.4574), $^1$H-NMR (500 M Hz, CDCl$_3$): $\delta$: 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), $^{13}$C-NMR (125 M Hz, CDCl$_3$): $\delta$: 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**

\(^1H\)-NMR (500 MHz, CDCl\(_3\)): \(\delta\): 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 (CH\(_2\)Cl\(_2\)/AcOEt solvent system), and fraction eluted with CH\(_2\)Cl\(_2\): AcOEt = 5:1 was collected. Further this fraction was rechromatographed (benzene/AcOEt solvent system), and fraction eluted with benzene: AcOEt = 10:1 afforded squamostanal-A (17) (2 mg) as an oil. MF: C\(_{18}\)H\(_{30}\)O\(_3\), MW: C\(_{18}\)H\(_{31}\)O\(_3\), State: oils, CD (MeOH) \(\Delta\varepsilon\) (nm): -0.42 (240), HR-FAB-MS: 295.2225 (Calcd for C\(_{18}\)H\(_{31}\)O\(_3\) 295.2273).

\(^1H\)-NMR (270 MHz, CDCl\(_3\)): \(\delta\): 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), 13C-NMR (67.5 MHz, CDCl\(_3\)): 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-15).

**Squamocin-C**

State: white crystals, MP: 50-51\(^{\circ}\). [\(\alpha\]D\(_{25}\)+19.5 \(\geq\) (c=0.92, MeOH). IR \(\nu_{max}\) (CHCl\(_3\)) cm\(^{-1}\): 3690, 3585, 3460, 1750. UV (MeOH) \(\lambda_{max}\) (nm): 210 (7000). CD (MeOH) \(\Delta\varepsilon\) (nm): -0.50 (240) HR-FAB-MS Calcd for C\(_{37}\)H\(_{67}\)O\(_7\) (MH\(^+\); m/z): 623.4887. Found: 623.4890.

\(^1H\)-NMR (500 MHz, CDCl\(_3\)): \(\delta\): 0.88 (3H, t, \(J=6.7\) Hz, H-34), 1.41 (3H, d, \(J=6.7\) 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, \(J=6.9\) Hz, H-36), 6.98 (1H, s, H-35).

**Squamocin-C triacetate**

FAB-MS \(m/z\): 749 (MH\(^+\)). \(^1H\)-NMR (500 MHz, CDCl\(_3\)): \(\delta\): 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**

\(^1H\)-NMR (500 MHz, CDCl\(_3\)): \(\delta\): 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, \(J=6.6\) Hz, H-15, 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).

**Squomocin-D**

A colorless oil. [\(\alpha\]D\(_{25}\)+30.1 \(\geq\) (c=0.58, MeOH). IR \(\nu_{max}\) (CHCl\(_3\)) cm\(^{-1}\): 3560, 3450, 1745. HR-FAB-MS Calcd for C\(_{37}\)H\(_{67}\)O\(_7\) (MH\(^+\); \(m/z\)): 623.4887. Found: 623.4830. \(^1H\)-NMR (500 MHz, CDCl\(_3\)): \(\delta\): 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), 13C-NMR (125 MHz, CDCl\(_3\)): 14.1 (C-34), 19.2 (C-37), 20.3 (C-15).
21.7 (C-26), 22.6 (C-33), 25.2 (C-3), 25.6 (C-13, -30), 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).

**Squamosin-D triacetate**

$^1$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 squamosin-D**

$^1$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. $[\alpha]_D^{25} +21.0$ (c=0.58, MeOH). IR $\nu_{max}$ (CHCl$_3$) cm$^{-1}$: 3560, 3450, 1750. HR-FAB-MS Calcd for C$_{37}$H$_{67}$O$_7$ (MH$^+$; m/z): 623.4887. Found: 623.4890. $^1$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), 2.40 (1H, dd, $J=15.0$, 8.2 Hz, H-3a), 2.52 (1H, br, $J=15.0$ Hz, H-3b), 3.38 (1H, m, H-15), 3.77-3.80 (3H, m, H-16, -24, -23), 3.80 (1H, m, H-12), 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).

**Squamosin-F triacetate**

$^1$H-NMR (500 MHz, CDCl$_3$) δ: 0.89 (3H, t, $J=6.4$ Hz, H-34), 1.41 (3H, d, $J=6.4$ Hz, H-37), 2.036, 2.074, 2.077 (3H each, s, A co), 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 squamosin-F**

$^1$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 $^\circ$ (from MeOH-water). $[\alpha]_D^{25} +28.5$ (c=0.50, MeOH). IR $\nu_{max}$ (CHCl$_3$) cm$^{-1}$: 3580, 3450, 1750. CD (MeOH) $\Delta\varepsilon$ (nm): -40 (240). Anal, Found: C, 71.64; H, 10.64. Calcd for C$_{37}$H$_{66}$O$_7$: C, 71.34; H, 10.68. $^1$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, $J=15.0$ 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), 13C-NMR (125 MHz, CDCl$_3$) δ: 14.0 (C-34), 19.0 (C-37), 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**

$^1$H-NMR (500 MHz, CDCl$_3$): $\delta$: 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, $J=5.9$ 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**

$^1$H-NMR (500 MHz, CDCl$_3$): $\delta$: 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, $J=6.4$ Hz, H-15), 5.06 (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 ̊C (from MeOH-water). $[\alpha]_{D}^{25}$ +21.8 (c = 0.61, MeOH). Anal. Found: C, 71.33; H, 10.87. Calcd for C$_{37}$H$_{66}$O$_7$: C, 71.34; H, 10.68. 1H-NMR (500 MHz, CDCl$_3$): $\delta$: 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, CDCl$_3$): $\delta$: 14.1 (C-34), 19.1 (C-37), 22.7 (C-33), 25.5 (C-6, -13), 25.6 (C-24), 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 ̊C (from MeOH-water). $[\alpha]_{D}^{25}$ +22.2 (c = 0.50, MeOH). IR $\nu_{\text{max}}$ (CHCl$_3$) cm$^{-1}$: 3560, 3450, 1750. UV $\lambda_{\text{max}}$ (MeOH) nm (log $\varepsilon$): 210 (3.7). CD (MeOH) $\Delta\varepsilon$ (nm): -0.29 (239) HR-FAB-MS Calcd for C$_{35}$H$_{63}$O$_6$: (MH$^+$; m/z): 579.4625. Found: 579.4640. 1H-NMR (500 MHz, CDCl$_3$): $\delta$: 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, CDCl$_3$): $\delta$: 14.1 (C-32), 19.2 (C-35), 25.6 (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, -30, -31), 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**

$^1$H-NMR: $\delta$: 0.88 (3H, t, $J=6.9$ Hz, H-32), 1.40 (3H, d, $J=6.6$ Hz, H-35), 2.045 (3H, s, A CO), 2.074 (3H, s, A CO), 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**
1H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, J = 7.0 Hz, H-32), 1.40 (3H, d, J = 6.9 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 = 6.8 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, M.P: 85-86.5°C (from MeOH-water), [α]D²⁵ +18.6 (c=0.42, MeOH). IR νmax (CHCl₃) cm⁻¹: 3560, 3450, 1750. UV λmax (MeOH) nm (log ε): 209 (3.8). CD (MeOH) De(D): -0.40 (241), Anal, Found: C, 73.08; H, 10.72. Calcd for C₃₅H₆₂O₆: C, 72.62; H, 10.80. 1H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, J = 7.0 Hz, H-32), 1.40 (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 = 6.8 Hz, H-34), 6.99 (1H, br s, H-33).

**Squamocin-J diacetate**

1H-NMR (500 MHz, CDCl₃) 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, m, 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**

1H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, J = 6.9 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, [α]D²⁵ +20.5 (c=0.53, MeOH). IR νmax (CHCl₃) cm⁻¹: 3560, 3450, 1750. Anal, Found: C, 72.34; H, 10.96. Calcd for C₃₅H₆₂O₆: C, 72.62; H, 10.80. 1H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, J = 6.9 Hz, H-32), 1.40 (3H, d, J = 6.5 Hz, H-35), 2.26 (2H, t, J = 7.7 Hz, H-3), 2.39 (2H, m, H-13, -22), 2.81-2.87 (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 = 7.0 Hz, H-33), 7.34-7.65 (10H, m, aromatic).

**Squamocin-K diacetate**

1H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, J = 6.5 Hz, H-35), 2.26 (2H, t, J = 7.7 Hz, H-3), 2.39 (2H, m, H-17, -18), 3.99 (2H, m, H-14, -21), 3.89 (2H, m, H-17, -18), 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**

1H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, J = 6.9 Hz, H-32), 1.40 (3H, d, J = 6.9 Hz, H-35), 2.26 (2H, m, H-17, -18), 2.95 (2H, m, H-13, -22), 4.86 (2H, m, H-14, -21), 4.99 (1H, q, J = 6.8 Hz, H-34), 6.98 (1H, br s, H-33).
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1H-NMR (500 MHz, CDCl₃) 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), 3.03 (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), 5.02 (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 °C (from MeOH-water). [α]D²⁵ +19.3 (c=0.98, MeOH). IR νmax (CHCl₃) cm⁻¹: 3590, 3450, 1745. Anal, Found: 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=6.9 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-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), 13C-NMR (125 MHz, CDCl₃) δ: 14.0 (C-34), 19.2 (C-37), 22.6 (C-33), 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), 83.3 (C-16), 134.3 (C-2), 148.8 (C-35), 173.8 (C-1).

Di-(R)-MTPA ester of squamocin-L
1H-NMR (500 MHz, CDCl₃) δ: 0.88 (3H, t, J=6.8 Hz, H-34), 1.41 (3H, d, J=6.9 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-15), 4.99 (1H, q, J=6.8 Hz, H-36), 6.98 (1H, s, H-35), 7.35-7.66 (15H, m, aromatic).

Squamocin-M
State: a colorless oil. [α]D²⁵ +26.0 (c=0.55, MeOH). Anal, Found: 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=6.9 Hz, H-34), 1.40 (3H, d, J=6.8 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). 13C-NMR (125 MHz, CDCl₃) δ: 14.1 (C-34), 19.2 (C-37), 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
1H-NMR (500 MHz, CDCl₃) 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 °C (from AcOEt). [α]D²⁵ +11.0 (c=0.40, MeOH). IR νmax (CHCl₃) cm⁻¹: 3680, 3590, 3400, 1750. UV λmax (MeOH) nm (ε): 210 (9300). CD (MeOH) δε (nm): -0.50 (240), 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, 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). 13C-NMR (125 MHz, CDCl₃) 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**

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 0.88 (3H, t, J=15.6, H-34), 1.41 (3H, d, J=5.8 Hz, H-34), 2.26 (2H, tt, J=7.7, 1.4 Hz, H-3), 3.471 (3H, s, OM-e), 3.490 (3H, s, OM-e), 3.535 (3H, s, OM-e), 3.578 (3H, s, OM-e), 3.67 (1H, m, H-20), 3.74 (1H, m, H-12), 3.89 (2H, m, H-15, -19), 4.91 (2H, m, H-16, -17), 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 $^\circ$ (from A COEt). $[\alpha]^{25}_D +10.5$ (c=0.10, MeOH). IR $\nu_{\text{max}}$ (CHCl$_3$) cm$^{-1}$: 3590, 3450, 1745. UV $\lambda_{\text{max}}$ (MeOH) nm (e): 209 (7000). CD (MeOH) $\Delta\varepsilon$(nm): -0.50 (238), HR-FA B-M S Calcd for C$_{37}$H$_{67}$O$_8$ (M H$^+$; m/z): 639.4836. Found: 639.4890. $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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). $^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta$: 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**

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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, OM-e), 3.495 (3H, s, OM-e), 3.523 (3H, s, OM-e), 3.580 (3H, s, OM-e), 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 $^\circ$ (from A COEt). $[\alpha]^{25}_D +12.0$ (c=0.10, MeOH). IR $\nu_{\text{max}}$ (CHCl$_3$) cm$^{-1}$: 3685, 3585, 3540, 1755. UV $\lambda_{\text{max}}$ (MeOH) nm (e): 210 (7000). CD (MeOH) $\Delta\varepsilon$(nm): -0.50 (238), HR-FA B-M S Calcd for C$_{37}$H$_{67}$O$_8$ (M H$^+$; m/z): 639.4836. Found: 639.4890. $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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). $^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta$: 14.1 (C-34), 19.1 (C-37), 22.7 (C-33), 25.5 (C-6), 26.1 (C-10), 28.4 (C-14), 28.7 (C-21), 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), 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), 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**

$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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, OM-e), 3.495 (3H, s, OM-e), 3.507 (3H, s, OM-e), 3.530 (3H, s, OM-e), 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 $^\circ$ (from MeOH-H$_2$O). $[\alpha]^{25}_D +7.9$ (c=0.51, MeOH). IR $\nu_{\text{max}}$ (CHCl$_3$) cm$^{-1}$: 3560, 3450, 1755. HR-FA B-M S Calcd for C$_{37}$H$_{67}$O$_7$ (M H$^+$; m/z): 623.4882. $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 0.89
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(3H, t, J = 5.9 Hz, H-34), 1.41 (3H, d, J = 6.7 Hz, H-37), 2.26 (2H, t, 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). 13C-NMR (125 MHz, CDCl3) 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, -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

1H-NMR (500 MHz, CDCl3) 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 = 7.7, 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). [α]D25 +14.7 > (c = 0.51, MeOH). IR νmax (CHCl3) cm⁻¹: 3560, 3450, 1750. Anal, Found: C, 71.64; H, 10.98. Calcd for C37H66O7: C, 71.34; H, 10.68. 1H-NMR (500 MHz, CDCl3) 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). 13C-NMR (125 MHz, CDCl3) δ: 14.1 (C-34), 19.2 (C-37), 22.7 (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, -30, -31), 31.9 (C-32), 32.4 (C-13), 33.4 (C-25), 35.6 (C-11), 74.5 (C-24), 74.2 (C-16 or -19), 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

1H-NMR (500 MHz, CDCl3) 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

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

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 holed 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 holed Nitsch & Nitsch (Nitsch and Nitsch, 1967) containing indole-3-acetic acid.
6. Upon subsequent inoculations, callus were cut to 5 mm diameter to petri dishes holed 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 grewed 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-dichrophenoxyacetic 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 disk. Germination was carried out in a moisture-saturated dark chamber for 72 hrs at 25 °C. Result 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.
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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 nuclei enhanced by polarization transfer
IR: infra red
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パンレイシ（Annona squamosa L.）種子に含まれる
パ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

パンレイシ科植物（annonaceae）は約130属2500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、その化学的成分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）あるいはパンレイシ科アセトゲニン類（annona-ceous 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ビ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。この図から明らかのように、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つの不斎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：パンレイシ、釣迦類）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）ではパ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Cl-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察されるが、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ンに適用した結果をFig.27に例示した。

この図から明らかのように、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察されるため、スクアモンの平面構造は一義的に決定できる。

本論文では、スクアモンの他に直結ビステトラヒドロフラン型のスクアモン-C、スクアモン-F、2つのテトラヒドロフラン環が4炭素で結ば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ション-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（スクアモン-O1、-O2、-N、-E、-B、スクアモスチン-Aおよびスクアモスタナール-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Moser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斎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three/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。