# Annonaceous Acetogenins from the Seeds of *Annona squamosa*. Adjacent Bis-tetrahydrofuranic Acetogenins

Mahendra SAHAI,*[a] Sanjewon SINGH,[a] Manorama SINGH,[a] Yogesh Kumar GUPTA,[a] Satoko AKASHI,[b] Reiko YUJI,[b] Kazuo HIRAYAMA,[b] Hitomi ASAKI,[c] Hiroshi ARAYA,[c] Noriyuki HARA,[c] Tadashi EGUCHI,[c] Katsumi KAKINUMA,[c] and Yoshinori FUJIMOTO*[c]

*Department of Medicinal Chemistry, I. M. S., Banaras Hindu University,[a] Varanasi 221005, India, Central Research Laboratories, Ajinomoto Co., Inc.,[b] Kawasaki 210, Japan, and Department of Chemistry, Tokyo Institute of Technology,[c] Meguro-ku, Tokyo 152, Japan.* Received October 12, 1993; accepted January 19, 1994

**The petroleum ether extract of the seeds of *Annona squamosa* L. yielded thirteen adjacent and four non-adjacent bis-tetrahydrofuranic acetogenins, in addition to squamocin (1) and squamostatin-A. The structures of the thirteen acetogenins, named as squamocins-B (2), -C (3), -D (4), -E (5), -F (6), -G (7), -H (8), -I (9), -J (10), -K (11), -L (12), -M (13) and -N (14), have been established on the basis of spectral evidence. Squamocins-B, -D, -E, -F, -I, -K and -N are new acetogenins. The structures of these acetogenins vary in the carbon number ($C_{37}$ or $C_{35}$), the number and position of hydroxyl groups (substituted at C-4, C-12, C-28 or C-29) and the stereochemistry at the bis-tetrahydrofuran moiety. Squamocin-N (14) has an unprecedented *threo–cis–threo–cis–threo* stereochemistry in its bis-tetrahydrofuran portion. Co-occurrence of a diastereoisomeric pair, 9 and 10, is noteworthy.**

**Keywords** annonaceous acetogenin; *Annona squamosa*; bis-tetrahydrofuran; squamocin; Annonaceae

A series of tetrahydrofuranic acetogenins has recently been isolated from certain genera of Annonaceae plants, *i.e.*, *Annona*, *Uvaria*, *Rollinia*, *Asimina* and *Goniothalamus*.[1)] These compounds, also referred to as annonaceous acetogenins, exhibit a variety of biological activities such as antitumor and pesticidal properties.[1)] *Annona squamosa* L. (Annonaceae) is well known for its edible tropical fruits (custard apples or sugar apples), and its seeds are reported to have insecticidal and abortifacient properties.[2)] As a part of our screening program for bioactive substances from tropical plants, we undertook a chemical investigation of the seeds of the plant and reported the isolation of squamocin (**1**)[3)] and squamostatin-A[4)] as cytotoxic principles. The earlier study showed the presence of a number of related compounds and we have now succeeded in the isolation of seventeen additional acetogenins from the seeds of *A. squamosa*. Among these, thirteen compounds, namely squamocins-B (**2**), -C (**3**), -D (**4**), -E (**5**), -F (**6**), -G (**7**), -H (**8**), -I (**9**), -J (**10**), -K (**11**), -L (**12**), -M (**13**) and -N (**14**) were established to be adjacent bis-tetrahydrofurans. In this paper, we describe the structure elucidation of **2**—**14** (Chart 1).

Annonaceous acetogenins can be classified into three types, *i.e.*, adjacent bis-tetrahydrofurans, non-adjacent bis-tetrahydrofurans, and mono-tetrahydrofurans.[1)] The acetogenins discussed in this paper all belong to the first type. The structures of the remaining four compounds, which belong to the second type, will be described in the following paper.

## Results and Discussion

In our earlier study, squamocin (**1**) and squamostatin-A were isolated from a waxy residue which precipitated from the petroleum ether extract of the ground seeds of *A. squamosa*. The supernatant portion of the extract was separated as follows. It was partitioned against aqueous MeOH. The MeOH layer was concentrated and the AcOEt-soluble part of the concentrate (*ca.* 8 g from 1 kg of seeds) was fractionated by silica gel chromatography into non-polar, moderately polar and polar fractions, with $CHCl_3$–AcOEt and then AcOEt–MeOH gradient systems as eluting solvents. The HPLC chromatogram of the

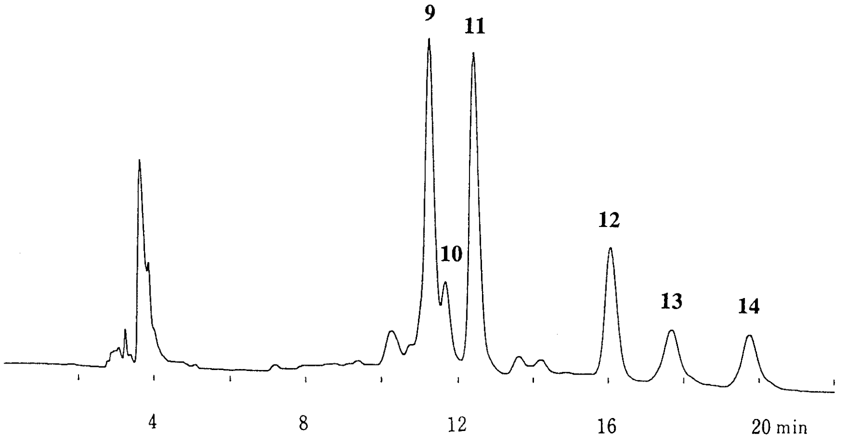

Fig. 1. HPLC Profile of Non-polar Fraction

Conditions: column, Shim-pack CLC-ODS (15 cm × 6 mm i.d.); solvent, MeOH–$H_2O$ (13 : 1); flow rate, 1.0 ml/min; UV detection at 220 nm.

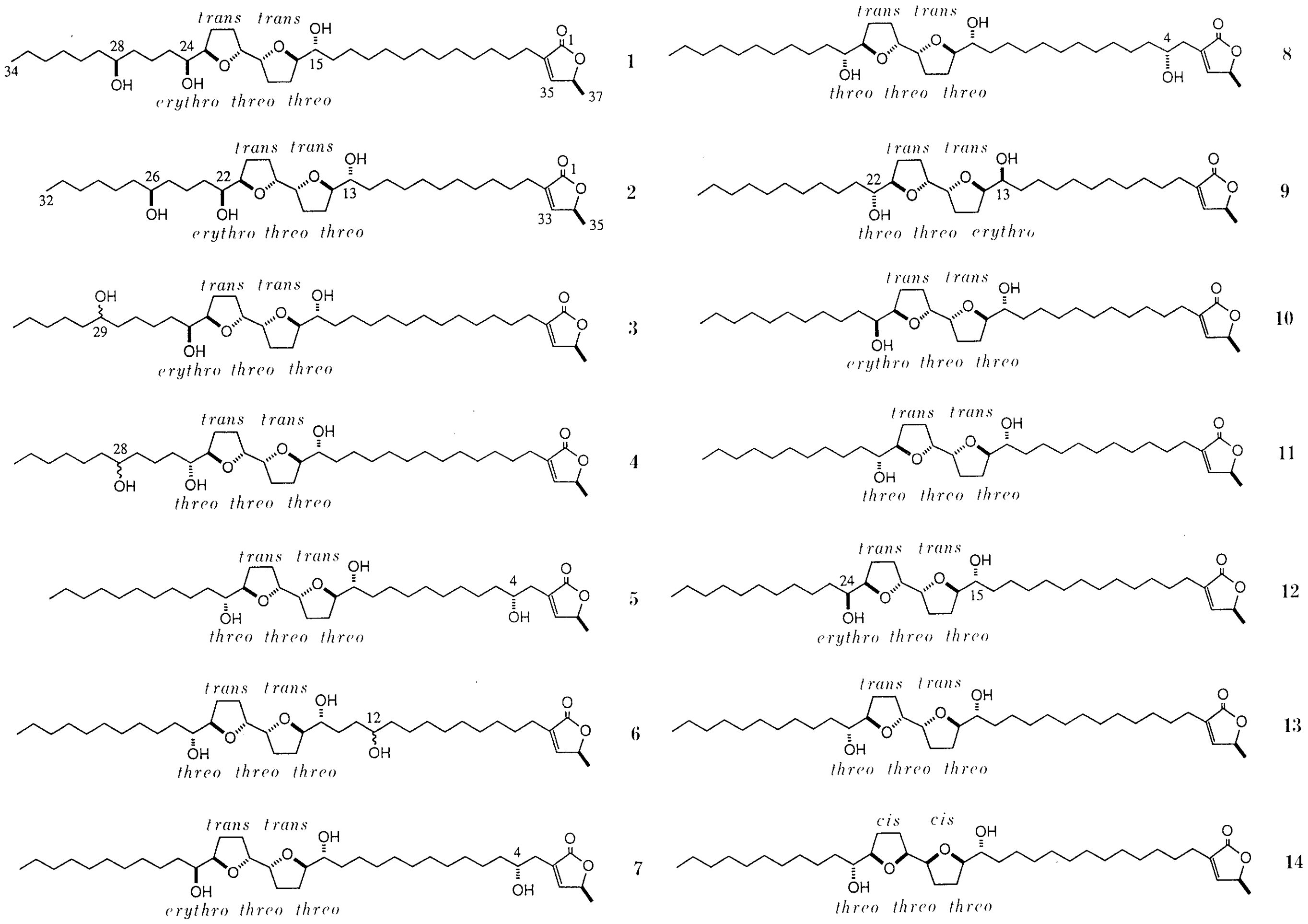

Chart 1. Structures of Adjacent Bis-tetrahydrofuranic Acetogenins (**1**—**14**)
The structures imply absolute stereochemistry.

non-polar fraction is illustrated in Fig. 1. Further separation of the non-polar fraction by reverse-phase octadecyl silica (ODS) HPLC with an MeOH–$H_2O$ system as the eluent furnished compounds **9**—**14**. Similarly, compounds **7** and **8** were isolated from the moderately polar fraction, while compounds **2**—**6** along with **1** and squamostatin-A were obtained from the polar fraction. Mobilities of **2**—**14** in HPLC and TLC are summarized in Table I.

In the previous paper on the structure elucidation of **1**,[3] we did not discuss the relative or absolute stereochemistry of **1**. Further, a number of $^{13}C$ signals of **1** were not precisely assigned. To obtain a more accurate $^{13}C$ assignment of **1**, the incredible natural abundance double quantum transfer experiment (INADEQUATE) spectrum of **1** was recorded. The spectrum clearly revealed the carbon–carbon connectivities from C-13 to C-18 and C-21 to C-30, thus affording the basis of a refined $^{13}C$ assignment (Table II). The signal assignments for C-19 and C-20 may still be interchangeable because the chemical shifts of C-18 and C-21 happened to be identical.

The absolute configurations at the chiral centers of squamocin (**1**) have been solved as follows. German researchers performed an X-ray study of a squamocin (annonin-I[5]) derivative and assigned 15*R*,16*R*,19*R*,20*R*,23*R*,24*S*,28*S*,36*S* relative stereochemistry (C-15/C-16-*threo*, C-16/C-19-*trans*, C-19/C-20-*threo*, C-20/C-23-*trans*, C-23/C-24-*erythro*).[6] We independently determined the C-36 configuration of **1** by chemical degradation. Thus,

Table I. Mobility in ODS-HPLC and Silica Gel TLC

| Compound | Retention time[a] | *Rf* value[b] | |
|---|---|---|---|
| Squamostatin-A | 7.8 (14.0) | 0.24 | |
| **2** | 8.9 (17.1) | 0.42 | |
| **3** | 10.7 (22.0) | 0.48 | |
| Squamocin (**1**) | 11.6 (25.5) | 0.44 | |
| **4** | 12.1 | 0.46 | |
| **5** | 12.2 | 0.50 | |
| **6** | 13.6 | 0.47 | |
| **7** | 14.6 | 0.59 | 0.10 |
| **8** | 16.7 | 0.59 | |
| **9** | 18.1 | | 0.40 |
| **10** | 18.7 | | 0.40 |
| **11** | 19.9 | | 0.36 |
| **12** | 25.4 | | 0.43 |
| **13** | 27.9 | | 0.46 |
| **14** | 30.8 | | 0.50 |

*a*) HPLC conditions: column, Shimadzu Shim-pack CLC-ODS (15 cm × 6 mm i.d.); solvent, MeOH–$H_2O$ (13 : 1); flow rate, 0.6 ml/min; UV detection at 220 nm. The retention times given in parentheses were recorded with MeOH–$H_2O$ (10 : 1) at the flow rate of 1.0 ml/min. *b*) Values in the left column were obtained on a Silica gel 60 $F_{254}$ plates (Merck) with $CHCl_3$–AcOEt–MeOH (10 : 5 : 1) as a developing solvent. Values in the right column were recorded with benzene–AcOEt (1 : 1) as a developing solvent.

two-step reduction of **1** with diisobutylaluminum hydride (DIBAH) and then $LiAlH_4$ gave the allylic alcohol, which was converted into the penta-(*R*)-α-methoxy-α-trifluoromethylphenylacetate (MTPA ester). Ozonolysis of the ester followed by Jones oxidation and diazomethane treatment furnished methyl lactate (*R*)-MTPA ester. The derivative was established to be the (2*S*)-antipode by comparison with authentic (2*R*)- and (2*S*)-specimens (TLC and GLC). It is thus concluded that squamocin (**1**) has 36*S* configuration. On the basis of the above findings, the absolute stereochemistry of **1** can be drawn as shown in the structure (**1**).

Squamocin exhibited a negative Cotton effect at 236 nm ($\Delta\varepsilon$, 0.45 in MeOH). With the established 36S configuration of **1**, this negative sign is attributable to the 36*S* configuration of **1**. Thus, the sign of the CD spectrum at about 240 nm can be unambiguously employed in the determination of the C-36 configuration of annonaceous acetogenins. The CD spectra of compounds **3**, **7**, **9**, **10** and **14** were measured and the 36*S* configuration has been determined for these compounds. Further, the CD data of acetogenins reported by other groups are consistent with this in terms of the negative sign at *ca.* 235—240 nm.[7,8] Thus, the structures of acetogenins **2**—**14** are depicted as having 36*S* (34*S*) configuration.

As far as the relative stereochemistry at the bis-tetrahydrofuran portion is concerned, three types of adjacent bis-tetrahydrofuranic acetogenins are known: (i) C-15/C-16-*threo*, C-16/C-19-*trans*, C-19/C-20-*threo*, C-20/C-23-*trans*, C-23/C-24-*erythro* type (*e.g.*, desacetyluvaricin[9] and squamocin[3]), (ii) *threo–trans–threo–trans–threo* type (*e.g.*, asimicin[10]), and (iii) *threo–cis–threo–cis–erythro* type (rollinastatin-1[11] being the sole example). These types can be readily distinguished by the method developed by Hoye and coworkers,[12] which is based on $^1$H-NMR comparison of the acetate derivatives of acetogenins.

Squamocin B (**2**) was isolated as a white wax. The molecular formula, $C_{35}H_{62}O_7$ [FAB-MS, *m/z* 595 ($MH^+$)], was two methylene units less than that of squamocin. The $^1$H- and $^{13}$C-NMR spectra of **2** were closely similar to those of **1**, showing a signal at $\delta_C$ 22.0, indicative of the presence of a 1,5-diol functionality. The mass spectrum of **2** (Fig. 2) exhibited a series of ion peaks starting with *m/z* 267 (C-13/C-14 cleavage), 337 (C-17/C-18 cleavage), 407 (C-21/C-22 cleavage) and 491 (C-26/C-27 cleavage $-H_2O$). These ions were shifted down by 28 mass units from the corresponding ions of squamocin and are consistent with the structure as depicted in Fig. 2.

The close similarity of the $^{13}$C-NMR data of **2** and **1** indicated the identity of the relative stereochemistry of the bis-tetrahydrofuran portion, *i.e.*, C-13/C-14-*threo*, C-14/C-17-*trans*, C-17/C-18-*threo*, C-18/C-21-*trans* and C-21/C-22-*erythro*. The H–H correlation spectroscopy (COSY) spectrum of the tri-(*R*)-MTPA ester of **2** revealed the following signal correlations: $\delta$ 5.14/3.87 and 5.02/3.99 (each set was coupled to the other) for two sets of $-C\underline{H}(OMTPA)-C\underline{H}(OR)-$, and $\delta$ 3.65/3.83 for $-C\underline{H}(OR)-C\underline{H}(OR)-$. The H-26 oxymethine signal resonated at $\delta$ 5.02. These chemical shifts and correlation pattern were essentially identical with those of **1**.[13] It is thus concluded that **2** has the aforementioned relative stereochemistry as well as 13*R*,14*R*,17*R*,18*R*,21*R*,22*S*,26*S* absolute configuration at the bis-tetrahydrofuran portion. On the basis of these data the structure of squamocin-B was established to be as shown in the formula (**2**).

Squamocin-C (**3**), $C_{37}H_{66}O_7$ [FAB-MS, *m/z* 623 ($MH^+$)], was isolated as white crystals, mp 50—51 °C. Compound **3** has three hydroxyl groups in the molecule as evidenced by the formation of the triacetate. Two of the hydroxyl groups were placed at C-15 and C-24, on either side of the bis-tetrahydrofuran portion ($\delta_H$ 3.39, $\delta_C$ 74.1 and $\delta_H$ *ca.* 3.9, $\delta_C$ 71.4), whereas the last one could be located along the hydrocarbon chain ($\delta_H$ 3.58, $\delta_C$ 71.9). The $^1$H-NMR spectrum of **3** was similar to that of **1**. However, the signals of C-26 to C-31 in the $^{13}$C-NMR of **3** were apparently different from those of **1**, suggesting that the last hydroxyl group might be present in the region near C-28, but not at C-28. The electron impact (EI)-MS of **3** (Fig. 3) was similar to that of **1** in the lower mass region, showing series of ion peaks starting with *m/z* 295 (C-15/C-16 cleavage), 365 (C-19/C-20 cleavage) and 417 (C-23/C-24 cleavage $-H_2O$). However, an enlarged spec-

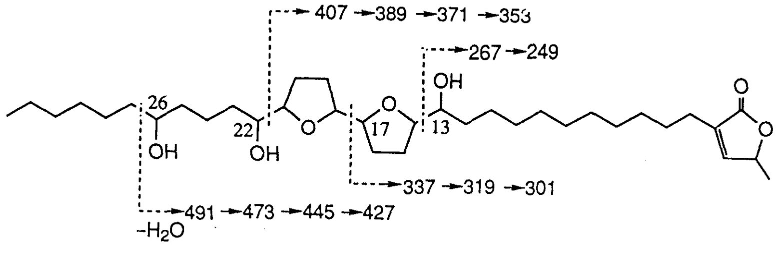

Fig. 2. Mass Fragmentation of Squamocin-B (**2**)

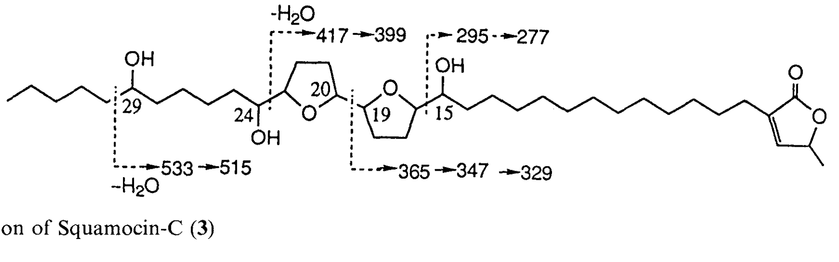

Fig. 3. Mass Fragmentation of Squamocin-C (**3**)

TABLE II. $^{13}$C-NMR Spectral Data for Adjacent Bis-tetrahydrofuranic Acetogenins (125 MHz, $CDCl_3$)

| C | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 173.9 | 173.9 | 173.9 | 173.9 | 174.6 | 173.9 | 174.5 | 174.6 | 173.8 | 173.9 | 173.8 | 173.8 | 173.8 | 173.9 |
| 2 | 134.3 | 134.3 | 134.4 | 134.3 | 131.1 | 134.3 | 131.1 | 131.2 | 134.3 | 134.3 | 134.3 | 134.3 | 134.3 | 134.4 |
| 3 | 25.2 | 25.1 | 25.2 | 25.2 | 33.3 | 25.1 | 33.2 | 33.3 | 25.2 | 25.2 | 25.1 | 25.1 | 25.1 | 25.2 |
| 4 | 27.4 | 27.4 | 27.4 | 27.4 | 69.9 | 27.4 | 69.9 | 70.0 | 27.3 | 27.4 | 27.3 | 27.4 | 27.4 | 27.4 |
| 5 | 29.2 | 29.1 | 29.2 | 29.2 | 37.3 | 29.1 | 37.3 | 37.3 | 29.1 | 29.2 | 29.1 | 29.1 | 29.1 | 29.2 |
| 6 | a) | a) | a) | a) | $25.5^{g)}$ | a) | $25.5^{j)}$ | $25.5^{l)}$ | a) | a) | a) | a) | a) | a) |
| 7—9 | a) | a) | a) | a) | a) | a) | a) | a) | a) | a) | a) | a) | a) | a) |
| 10 | a) | a) | a) | a) | a) | 25.7 | a) | a) | a) | a) | a) | a) | a) | a) |
| 11 | a) | 25.6 | a) | a) | $25.5^{g)}$ | 37.6 | a) | a) | 26.0 | 25.7 | 25.6 | a) | a) | a) |
| 12 | a) | 33.2 | a) | a) | 33.4 | 71.7 | a) | a) | 32.4 | 33.4 | 33.4 | a) | a) | a) |
| 13 | 25.7 | 74.1 | 25.7 | 25.6 | 74.0 | 33.5 | $25.7^{j)}$ | $25.6^{l)}$ | 71.3 | 74.1 | 74.0 | 25.6 | 25.6 | 25.8 |
| 14 | 33.3 | 83.3 | 33.4 | 33.4 | 83.1 | a) | 33.3 | 33.5 | 82.8 | 83.3 | 83.1 | 33.3 | 33.5 | 34.4 |
| 15 | 74.1 | 28.4 | 74.1 | 74.1 | 28.3 | 74.2 | 74.2 | 74.1 | 24.4 | 28.4 | 28.4 | 74.1 | 74.0 | 74.0 |
| 16 | 83.3 | 28.9 | 83.3 | $83.1^{e)}$ | 28.9 | 83.2 | 83.3 | 83.1 | 28.9 | 28.9 | 28.9 | 83.3 | 83.1 | 82.8 |
| 17 | 28.4 | $82.2^{c)}$ | 28.4 | 28.4 | 81.7 | $28.3^{h)}$ | 28.3 | 28.3 | $82.5^{m)}$ | $82.3^{n)}$ | 81.7 | 28.4 | 28.5 | 29.0 |
| 18 | 28.9 | $82.5^{c)}$ | 28.9 | $28.9^{f)}$ | 81.7 | 28.9 | 28.9 | 28.9 | $82.2^{m)}$ | $82.5^{n)}$ | 81.7 | 29.0 | 28.9 | 27.3 |
| 19 | $82.2^{b)}$ | 28.9 | $82.2^{d)}$ | 81.8 | 28.9 | $81.8^{i)}$ | $82.3^{k)}$ | 81.7 | 28.9 | 29.0 | 28.9 | $82.2^{o)}$ | 81.7 | 81.0 |
| 20 | $82.5^{b)}$ | 24.8 | $82.5^{d)}$ | 81.8 | 28.3 | $81.7^{i)}$ | $82.6^{k)}$ | 81.7 | 28.4 | 24.5 | 28.4 | $82.5^{o)}$ | 81.7 | 81.0 |
| 21 | 28.9 | 82.8 | 28.9 | $29.0^{f)}$ | 83.1 | 28.9 | 28.9 | 28.9 | 83.3 | 82.8 | 83.1 | 29.1 | 28.9 | 27.3 |
| 22 | 24.8 | 71.4 | 24.6 | 28.4 | 74.0 | $28.4^{h)}$ | 24.4 | 28.3 | 74.1 | 71.4 | 74.0 | 24.4 | 28.5 | 29.0 |
| 23 | 82.8 | 32.4 | 82.8 | $83.2^{e)}$ | 33.4 | 83.2 | 82.8 | 83.1 | 33.2 | 32.5 | 33.4 | 82.8 | 83.1 | 82.8 |
| 24 | 71.4 | 22.0 | 71.4 | 73.9 | $25.6^{g)}$ | 74.0 | 71.3 | 74.1 | 25.5 | 26.0 | 25.6 | 71.5 | 74.0 | 74.0 |
| 25 | 32.5 | 37.2 | 32.4 | 33.2 | a) | 33.4 | 32.4 | 33.5 | a) | a) | a) | 32.4 | 33.5 | 34.4 |
| 26 | 22.0 | 71.7 | 26.1 | 21.7 | a) | 25.6 | 26.0 | $25.5^{l)}$ | a) | a) | a) | 26.0 | 25.6 | 25.8 |
| 27 | 37.3 | 37.4 | 25.7 | 37.3 | a) | a) | a) | a) | a) | a) | a) | a) | a) | a) |
| 28 | 71.8 | 25.6 | 37.5 | 71.7 | a) | a) | a) | a) | a) | a) | a) | a) | a) | a) |
| 29 | 37.5 | a) | 71.9 | 37.5 | a) | a) | a) | a) | a) | a) | a) | a) | a) | a) |
| 30 | 25.7 | 31.8 | 37.3 | 25.6 | 31.9 | a) | a) | a) | 31.9 | 31.9 | 31.9 | a) | a) | a) |
| 31 | 29.7 | 22.6 | 25.3 | 29.7 | 22.6 | a) | a) | a) | 22.6 | 22.7 | 22.6 | a) | a) | a) |
| 32 | 31.8 | 14.0 | 31.9 | 31.8 | 14.1 | 32.0 | 31.8 | 31.9 | 14.0 | 14.1 | 14.0 | 31.9 | 31.9 | 31.9 |
| 33 | 22.6 | 148.8 | 22.6 | 22.6 | 151.8 | 22.8 | 22.6 | 22.7 | 148.8 | 148.8 | 148.8 | 22.6 | 22.6 | 22.7 |
| 34 | 14.1 | 77.4 | 14.0 | 14.1 | 77.9 | 14.2 | 14.0 | 14.1 | 77.3 | 77.4 | 77.3 | 14.0 | 14.1 | 14.1 |
| 35 | 148.8 | 19.2 | 148.8 | 148.8 | 19.1 | 149.0 | 151.7 | 151.7 | 19.2 | 19.2 | 19.2 | 148.8 | 148.8 | 148.8 |
| 36 | 77.4 | — | 77.4 | 77.4 | — | 77.5 | 77.9 | 77.9 | — | — | — | 77.3 | 77.3 | 77.4 |
| 37 | 19.2 | — | 19.2 | 19.2 | — | 19.3 | 19.0 | 19.1 | — | — | — | 19.2 | 19.2 | 19.2 |

*a*) The signals overlapped in the region of $\delta$ 29—30. *b*—*o*) Assignments may be interchanged within the column.

trum showed ion peaks at *m*/*z* 533 (C-30/C-29 fission $-H_2O$, 0.6% of base peak ion, 295), 515 (533-$2H_2O$, 1.1%) and 497 (533-$3H_2O$, 1.0%) in the higher mass region. These ions were shifted up by 14 mass units from those of squamocin. Thus, the position of the hydroxyl group was settled as C-29.

We were initially perplexed by the $^{13}$C-NMR spectra of **3**. It was expected that the $^{13}$C-NMR spectrum of **3** would exhibit six signals (C-3, -13, -21, -26, -27, -31) in the $\delta$ 24—27 region, and four signals (C-14, -25, -28, -30) in the $\delta$ 32—38 region. However, the $^{13}$C-NMR of **3**, recorded in $CDCl_3$, exhibited only five signals ($\delta$ 24.6, 25.2, 25.3, 25.7, 26.1) in the former region and four signals ($\delta$ 32.4, 33.4, 37.3, 37.5) in the latter region. Even when the spectrum was recorded in $CD_3OD$, five signals ($\delta$ 26.0, 26.5, 26.9, 27.0, 27.3) and three signals ($\delta$ 34.1, 34.4, 38.2) were observed in the respective regions. The signals underlined appeared to be due to two carbons. The assignments of C-29 to C-33 (Table II) followed the $^{13}$C data for 6-hydroxy-undecane.

The close similarity in the $^{13}$C chemical shifts of the bis-tetrahydrofuran portion of **3** and **1** indicated their stereochemical identity at this region. This was further supported by the $^{1}$H-NMR spectrum of the triacetate of **3**, which exhibited signals at $\delta$ 3.90 (H-19, -20), 3.98 (H-16, -23) and 4.82—4.92 (H-15, -24), among others. The chemical shifts of these oxymethine signals were consistent with C-15/C-16-*threo*, C-16/C-19-*trans*, C-19/C-20-*threo*, C-20/C-23-*trans*, C-23/C-24-*erythro* structure (Table III).[12]

The $^{1}$H-NMR spectrum of the tri-(*R*)-MTPA ester of **3** exhibited signals at $\delta$ 3.90/5.16 (H-23/H-24), 3.99/5.04 (H-15/H-16), 3.66/3.83 (H-19/20) and 5.04 (H-29). Since the chemical shifts of these signals resembled those of the (*R*)-MTPA esters of **1**, the absolute configuration of the bis-tetrahydrofuran portion was deduced to be 15*R*,16*R*,19*R*,20*R*,23*R*,24*S*. The configuration of C-29 remained to be elucidated. On the basis of the data presented above, the structure of **3** was established to be as shown in the formula (**3**). Squamocin-C was identical with motrilin,[14] recently isolated from *A. cherimolia*, based on a comparison of the spectral data with the published values ($^{13}$C-NMR, MS, as well as melting point). This is the first report of isolation of squamocin-C from *A. squamosa*.

Squamocin D (**4**), $C_{37}H_{66}O_7$ [FAB-MS, *m*/*z* 623 ($MH^+$)], was obtained as a viscous oil. The $^{13}$C-NMR data for **4** indicated that it is an adjacent bis-tetrahydrofuranic acetogenin ($\delta_C$ 73.9, 74.1, 81.8, 81.8, 83.1, 83.2) bearing one hydroxyl group along the hydrocarbon chain ($\delta_C$ 71.7), in addition to the two hydroxyl groups adjacent to the tetrahydrofuran ring. The $^{13}$C-NMR spectrum also displayed a signal at $\delta$ 21.7, suggesting the presence of a 1,5-diol system. The EI-MS of **4** (Fig. 4) was similar to that of squamocin, including fragment ion peaks at $m/z$ 519 and 501 due to fission at C-28/C-29 followed by dehydration. These data suggested that **4** is a stereoisomer of **1**.

In contrast to the C-15/C-16-*threo*, C-23/C-24-*erythro* structure of squamocin (**1**), C-15/C-16-*threo*, C-23/C-24-*threo* structure was readily presumed for **4** on the basis of the chemical shifts (Born's rule [6]) of the oxymethine proton and carbon signals alpha to the tetrahydrofuran ring ($\delta_H$ *ca.* 3.85/$\delta_C$ 73.9 and $\delta_H$ *ca.* 3.85/$\delta_C$ 74.1). The $^1$H-NMR data for the triacetate of **4** further established *threo–trans–threo–trans–threo* structure of **4** on the basis of the chemical shifts of H-19/H-20, H-16/H-23 and H-15/H-24, which are in good agreement with those of such an isomer (Table III). Although the oxymethine proton signals in the $^1$H-NMR spectrum of the tri-($R$)-MTPA ester of **4** were overlapped to some extent, the H–H COSY spectrum allowed us to assign the signals due to a –C$\underline{H}$(OMTPA)–C$\underline{H}$(OR)– unit at $\delta$ 5.03/3.99. The $\delta$ 5.03/3.99 signals were also observed in the $^1$H-NMR spectra of the ($R$)-MTPA esters of **1**, **2** and **3**. Therefore, the set of signals was assigned to H-15 and H-16, but not H-24 and H-23. It seems reasonable to assume a 15$R$,16$R$ (therefore, 15$R$,16$R$,19$R$,20$R$,23$R$,24$R$) configuration for **4** on the basis of the chemical shifts of the above set of signals. Thus, the structure of **4** was established to be as shown in the formula (**4**). Squamocin-D is a new acetogenin.

TABLE III. Chemical Shifts of Oxymethine Protons of Bis-tetrahydrofuranic Acetogenin Acetates (500 MHz, $CDCl_3$)

| Stereoisomers | H-15 (H-24) | H-16 (H-23) | H-19 (H-20) |
|---|---|---|---|
| **4** (*threo–trans–threo–trans–threo*) | 4.86 | 3.99 | 3.91 |
| **5** (*threo–trans–threo–trans–threo*)[a] | 4.86 | 3.99 | 3.91 |
| **6** (*threo–trans–threo–trans–threo*) | 4.85 | 3.99 | 3.90 |
| **11** (*threo–trans–threo–trans–threo*)[a] | 4.86 | 3.99 | 3.91 |
| **14** (*threo–cis–threo–cis–threo*) | 4.95 | 3.94 | 3.87 |
| **3** (*threo–trans–threo–trans–erythro*) | 4.82—4.92[b] | 3.98 | 3.90 |
| | | (3.98) | (3.90) |
| **9** (*threo–trans–threo–trans–erythro*)[a] | 4.91 | 3.98 | 3.89 |
| | (4.86) | (3.98) | (3.89) |
| **10** (*threo–trans–threo–trans–erythro*)[a] | 4.86 | 3.98 | 3.89 |
| | (4.91) | (3.98) | (3.89) |
| *threo–trans–threo–trans–erythro*[c] | 4.86 | 3.97 | 3.90 |
| | (4.92) | (3.98) | (3.89) |
| *threo–trans–threo–trans–threo*[c] | 4.86 | 3.97 | 3.90 |
| *threo–trans–erythro–trans–threo*[c] | 4.84 | 3.97 | 3.83 |
| *threo–cis–threo–cis–threo*[c] | 4.94 | 3.93 | 3.86 |
| *threo–cis–erythro–cis–threo*[c] | 4.84 | 3.92 | 3.77 |

*a*) The chemical shifts of H-13 (H-22), H-14 (H-21) and H-17 (H-18). *b*) The H-29 signal was overlapped. *c*) Model compound acetates, adopted from ref. 12.

Squamocin-E (**5**), $C_{35}H_{62}O_7$ [FAB-MS, $m/z$ 595 ($MH^+$)], was isolated as white crystals, mp 48—50 °C. This is a $C_{35}$ adjacent bis-tetrahydrofuranic acetogenin bearing three hydroxyl groups ($\delta_C$ 69.9, 74.0, 74.0). The oxymethine carbon signal at $\delta$ 69.9 was reminiscent of C-4 hydroxylated acetogenins.[10] The C-4 hydroxy function was also supported by the $^1$H-NMR spectrum of **5**, in which the C-3 methylene protons were observed as a part of a characteristic ABX system at $\delta$ 2.40 and 2.53.[10]

The EI-MS of **5** exhibited fragment ion peaks starting with $m/z$ 283 (C-13/C-14 cleavage), 353 (C-17/C-18 cleavage) and 423 (C-21/C-22 cleavage). The mass spectral data (Fig. 5) were consistent with the structure depicted in Fig. 5, in which the methylene chain linking the lactone and bis-tetrahydrofuran portions is shortened by two methylene units as compared with usual $C_{37}$ acetogenins.

The stereochemistry of the bis-tetrahydrofuran portion was determined to be *threo–trans–threo–trans–threo* on the basis of the $^1$H-NMR data for the triacetate of **5** (Table III). The absolute stereochemistry at C-4 and C-34 of **5** was determined as $R$ and $S$, respectively, on the basis of the $^1$H-NMR data for the tri-($R$)-MTPA ester (compare the data with those for **7**).

The absolute stereochemistry of the tetrahydrofuran portion of **5** was determined to be 15$R$,16$R$,18$R$,20$R$,23$R$,24$R$, since the $^1$H-NMR data for the tri-($R$)-MTPA ester of **5** [$\delta$ 3.93 (H-17, -18), 4.00 (H-14, -21) and 5.03 (H-13, -22)] were in complete agreement with those of the corresponding ester of asimicin. The 15$R$,16$R$,18$R$,20$R$,

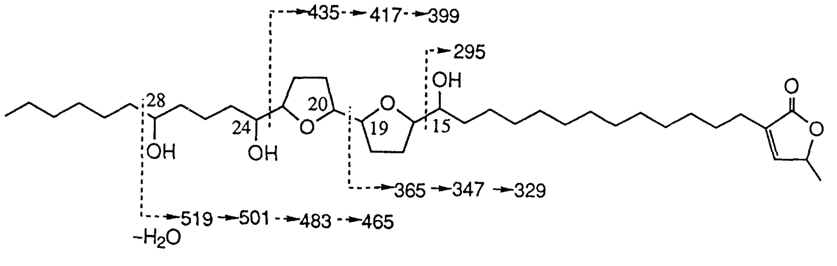

Fig. 4. Mass Fragmentation of Squamocin-D (**4**)

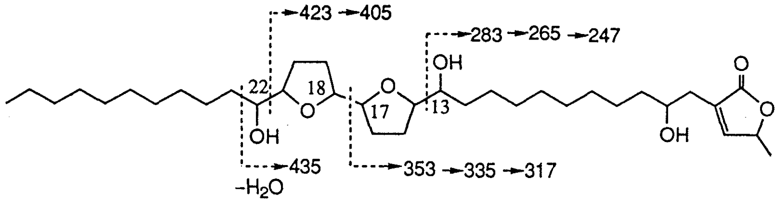

Fig. 5. Mass Fragmentation of Squamocin-E (**5**)

23*R*,24*R* configuration of asimicin has been established by the application of the advanced Mosher method.[15] Thus, the structure of squamocin-E was established to be as depicted in the formula (**5**). This is a new acetogenin.

Squamocin-F (**6**), $C_{37}H_{66}O_7$, was obtained as a white wax and found to have one hydroxyl group ($\delta_H$ 3.60 and $\delta_C$ 71.7) along the hydrocarbon chain, in addition to two hydroxyl groups adjacent to the tetrahydrofuran ring ($\delta_C$ 74.0, 74.2). The EI-MS of **6** exhibited series of ion peaks starting with *m/z* 293 (C-15/C-16 cleavage$-H_2O$), 363 (C-19/C-20 cleavage$-H_2O$)) and 433 (C-23/C-24 cleavage$-H_2O$) (Fig. 6). The fragmentation pattern indicated that the additional hydroxyl group is located between the bis-tetrahydrofuran portion and the lactone ring. However, the spectrum did not show significant fragment ions which would afford conclusive evidence for assignment of the hydroxyl position (an ion at *m/z* 253 corresponding to C-12/C-13 cleavage was observed at a level close to the noise level).

This problem was settled by the application of a precursor-ion scanning method for an amine derivative (the structure of the derivative is illustrated in Fig. 7) of **6**.[16] Precursor ions which produce *m/z* 72 as a product ion were scanned. As can be seen in Fig. 7, the spectrum clearly exhibited ion peaks at *m/z* 293 and 323, corresponding to the ions formed by C-11/C-12 and C-12/C-13 fission followed by dehydration, respectively. Therefore, the hydroxyl group was unambiguously assigned at C-12.

Consideration of the $^{13}C$ chemical shifts alpha to a hydroxyl group further supported the above assignment. Three such signals were present in the $^{13}C$-NMR spectrum of **6** and could be assigned to C-11 ($\delta$ 37.6), C-13 ($\delta$ 33.5) and C-25 ($\delta$ 33.4, *e.g.*, see C-25 of **13** and C-23 of **11**). The signal of C-14 is most likely to be buried in the region of methylene overlap ($\delta$ 29—30). The chemical shift of C-13 was shifted upfield by *ca.* $\delta$ 4 from the value expected for the carbon alpha to a hydroxyl group. The gap can be explained by assuming a beta-effect of a hydroxyl group in the $^{13}C$ chemical shift. This implies that **6** has a 1,4-diol system, *i.e.*, 12,15-diol structure, in the molecule.

The stereochemistry of the bis-tetrahydrofuran portion

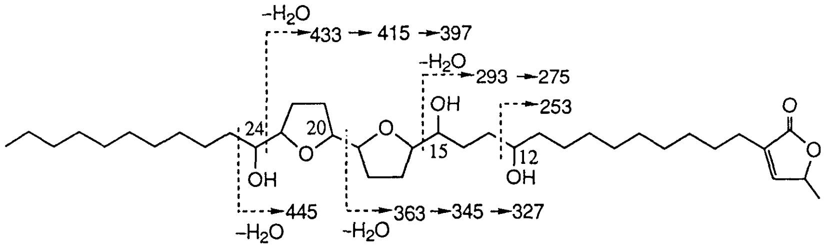

Fig. 6. Mass Fragmentation of Squamocin-F (**6**)

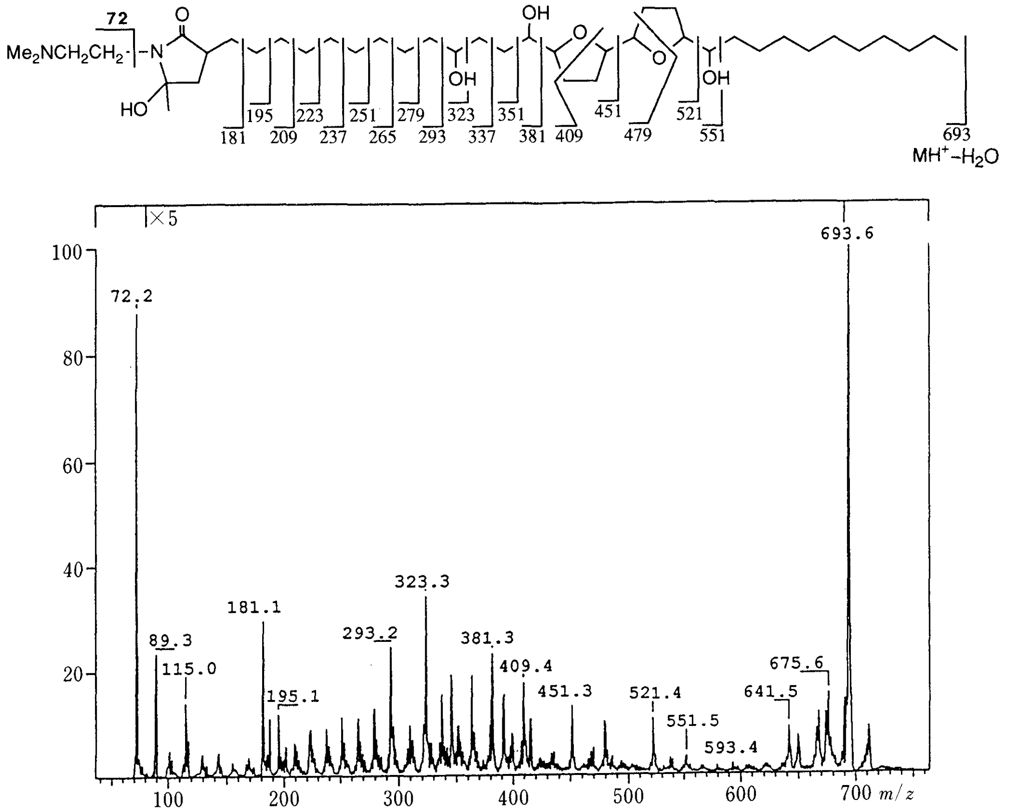

Fig. 7. Precursor Ion Scanning Spectrum from *m/z* 72 of the *N*,*N*-Dimethylethylenediamine Derivative of Squamocin-F (**6**)

The mass number indicated in the structure corresponds to the ion formed by fission at the indicated position followed by elimination of water.

was suggested to be *threo–trans–threo–trans–threo* on the basis of the close similarity of the $^{13}$C-NMR data of **6** and **4**. This was confirmed by analysis of $^{1}$H-NMR data for the triacetate of **6** (Table III). Since the oxymethine proton region was poorly resolved in the $^{1}$H-NMR spectrum of the tri-(*R*)-MTPA ester of **6** [$\delta$ 3.89—3.94 (H-19, -20), 3.94—4.02 (H-16, -23), 4.90—5.05 (H-12, -15, -24, -36)], it was difficult to obtain a conclusive answer regarding the absolute stereochemistry on the bis-tetrahydrofuran portion. We, however, presume 15*R*,16*R*,19*R*,20*R*,23*R*,24*R* configuration by analogy with the other acetogenins presented in this paper. Thus, the structure of squamocin-F was elucidated to be as shown in the formula **6**. Squamocin-F is a novel acetogenin and the first example of an adjacent bis-tetrahydrofuran bearing a 12-hydroxyl function.

Squamocin-G (**7**), $C_{37}H_{66}O_7$ [FAB-MS, *m/z* 623 (MH$^+$)], was isolated as white crystals, mp 77—78 °C. The chemical shift of C-4 ($\delta$ 69.9) in the $^{13}$C-NMR and the characteristic ABX pattern of C-3 methylene protons in the $^{1}$H-NMR indicated that **7** is a C-4 hydroxylated acetogenin. Series of ion peaks starting with *m/z* 311 (C-15/C-16 cleavage), 381 (C-19/C-20 cleavage) and 451 (C-23/C-24 cleavage) were observed in the EI-MS of **7**, which was in accordance with the structure depicted in Fig. 8. *Threo–trans–threo–trans–erythro* structure[17] at the bis-tetrahydrofuran portion of **7** was deduced from a comparison of the $^{13}$C-NMR data for **7** with those for squamocin and squamocin-C. $^{1}$H-NMR data for (*R*)- and (*S*)-MTPA esters of *threo–trans–threo–trans–erythro* type acetogenins with a defined absolute configuration have recently been reported.[15] Comparison of these $^{1}$H-NMR data with those of **7** allowed to established that **7** has 15*R*,16*R*,19*R*,20*R*,23*R*,24*S* configuration. The CD spectrum of **7** showed a negative Cotton effect at 240 nm, thus establishing 36*S* configuration.

As a representative of C-4 hydroxylated acetogenins (compounds **5**, **7** and **8** in the present work), the C-4 stereo-chemistry of **7** was investigated by applying the advanced Mosher method.[18] The pertinent $^{1}$H-NMR data for the (*R*)- and (*S*)-MTPA esters are listed in Table IV, clearly establishing the 4*R*-configuration of **7**. On the basis of the above considerations, the structure of squamocin-G was established as shown in the formula (**7**).

Concerning the C-4 configuration, the same result using the same method has been reported by Rieser *et al.*[15] The configuration at C-4 and C-36 was now readily determined by comparing the $^{1}$H-NMR data for the corresponding (*R*)-MTPA ester with the data listed in Table IV. In this way 4*R*,36*S* configuration was also assigned for compounds **5** and **8**.

Squamocin-G appears to be identical with bullatacin[7] (mp 69—70 °C), isolated from *A. bullata* and rollinastatin-2[19] (mp 73—76 °C) from *Rollina mucosa*. A bis-tetrahydrofuranic acetogenin named 14-hydroxy-25-deoxyrollinicin[20] (mp 68—70 °C, structure revision was suggested in ref. 1) from *A. reticulata* also appears to be identical with **7**. Isolation of rollinastatin-2 from *A. cherimolia*,[14] and bullatacin from *A. squamosa*,[21] *A. reticulata*,[22] *A. triloba*[23] and *A. purpurea*[24] was also reported. It should be emphasized that identification of these compounds cannot be attained by spectral comparison (see the section on squamocins-I and J).

Squamocin-H (**8**), $C_{37}H_{66}O_7$, MW 622, was isolated as white crystals, mp 45—48 °C. The identity of **8** as asimicin (mp 68—69 °C), isolated from *Asimina triloba*,[10] *A. cherimolia*,[25] *A. squamosa*[6] and *A. glabra*,[26] was established on the basis of spectral comparison. The 15*R*,16*R*,19*R*,20*R*,23*R*,24*R* configuration has been reported for asimicin based on the results of the advanced Mosher method.[15] The absolute configuration of such symmetrical type bis-tetrahydrofuranic acetogenins can now be determined by the simple comparison of the $^{1}$H-NMR data of their (*R*)-MTPA ester with those of **8**. We were thus able to assign 15*R*,16*R*,19*R*,20*R*,23*R*,24*R* configuration for **4**, **5**, **11** and **13**.

Squamocin-I (**9**) was isolated as white crystals, mp 68.5—71 °C. The compound was found to be a $C_{35}$ bis-tetrahydrofuranic acetogenin having only two hydroxyl groups [molecular formula, $C_{35}H_{62}O_6$ (FAB-MS, *m/z* 579 (MH$^+$)]. The $^{1}$H- and $^{13}$C-NMR data for the bis-tetrahydrofuran portion of **9** were typical of *threo–trans–threo–trans–erythro* structure. This was confirmed by analysis of the $^{1}$H-NMR spectrum of the diacetate of **9** (Table III). The EI-MS spectrum of **9** showed intense ion peaks at *m/z* 267 (fission at C-13/C-14), 337 (fission at

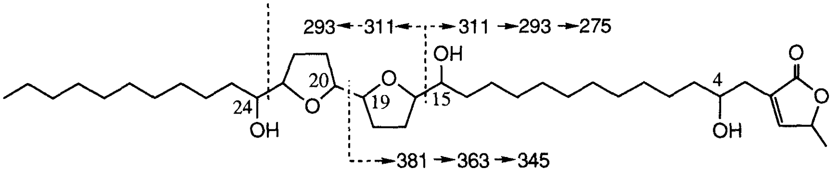

Fig. 8. Mass Fragmentation of Squamocin-G (7)

Table IV. $^{1}$H-NMR (500 MHz, $CDCl_3$) Data for the (*R*)- and (*S*)-MTPA Ester Derivatives of Squamocin-G (7)

| | 3-H$_a$ | 3-H$_b$ | H-4 | 35-H | 36-H | 37-H$_3$ | 5-H$_{ab}$$^{a)}$ |
|---|---|---|---|---|---|---|---|
| (*R*)-Ester | 2.59 | 2.68 | 5.38 | 6.97 | 4.90 | 1.31 | *ca.* 1.56, 1.65 |
| (*S*)-Ester | 2.58 | 2.58 | 5.32 | 6.73 | 4.86 | 1.28 | *ca.* 1.63, 1.69 |
| $\Delta_{(S)-(R)}$ | −0.01 | −0.10 | −0.06 | −0.24 | −0.04 | −0.03 | +0.07 +0.04 |

*a*) The chemical shifts of these signals were estimated from the H–H COSY spectra.

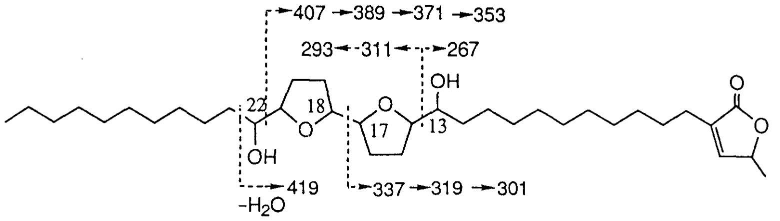

Fig. 9. Mass Fragmentation of Squamocin-I (**9**)

TABLE V. $^1$H-NMR Data for the ($R$)- and ($S$)-MTPA Ester Derivatives of Squamocin-N (**14**) (500 MHz, $CDCl_3$)

| | 14/25-$H_{ab}$[a] | | 15/24-H | 16/23-H | 19/20-H |
|---|---|---|---|---|---|
| ($R$)-Ester | 1.49 | 1.54 | 5.49 | 3.90 | 3.90 |
| ($S$)-Ester | 1.63 | 1.66 | 5.16 | 3.95 | 3.76 |
| $\Delta_{(S)-(R)}$ | +0.14 | +0.12 | −0.33 | +0.05 | −0.14 |

[a] The chemical shifts of these signals were estimated from the H–H COSY spectra.

C-17/C-18) and 407 (fission at C-21/C-22$-H_2O$). These spectral data are consistent with the structure depicted in Fig. 9. The CD spectrum of **9** showed a negative Cotton effect at 239 nm, indicating 34$S$ configuration. Squamocin-J (**10**), white crystals, mp 85—86.5 °C, was slightly less mobile than **9** on ODS-HPLC. Compound **10** resembled **9** in the EI-MS as well as $^1$H- and $^{13}$C-NMR data (the maximum difference in the chemical shifts was $\delta$ 0.19 for C-12/C-23 in $^{13}$C-NMR). Further, the $^1$H-NMR data for the diacetate derivative of **10** were again in close agreement with those for the diacetate of **9** (the maximum difference in the chemical shifts was $\delta$ 0.002 for one of $CH_3COO$), suggesting *threo–trans–threo–trans–erythro* stereochemistry for the bis-tetrahydrofuran portion (Table V). The CD spectrum of **10** was also essentially identical with that of **9**, indicating 34$S$ stereochemistry. The $^1$H-NMR spectrum of the di-($R$)-MTPA ester was again closely similar to that of **9** (the maximum difference in the chemical shifts was $\delta$ 0.02 for one of $CH_3O$).

This unique situation could be rationally explained only by assuming 13$S$,14$R$,17$R$,18$R$,21$R$,22$R$ and 13$R$,14$R$,17$R$,18$R$,21$R$,22$S$ stereochemistry for the two compounds (although we could not predict which of **9** and **10** corresponds to which of the two isomers; for convenience, **9** is tentatively referred to as 13$S$,14$R$,17$R$,18$R$,21$R$,22$R$ in this paper). This is the first time that such a pair of acetogenins has been isolated. The present identification of both **9** and **10** shows that care is necessary in the structure determination and identification of annonaceous acetogenins, in particular *threo–trans–threo–trans–erythro* type compounds. At present, direct HPLC comparison appears to be the only method available for identification.

Kawazu and coworkers reported the isolation of neoannonin, a $C_{35}$ *threo–trans–threo–trans–erythro* bis-tetrahydrofuranic acetogenin, from *A. squamosa* seeds.[27] Direct comparison of the $^{13}$C-NMR spectrum of neoannonin with those of **9** and **10** suggested the identity of **10** and neoannonin. Neoannonin reported by Kawazu *et al.* seems to be a major acetogenin. In our case, compound **10** was present in a much smaller amount than **9**, as can be seen in Fig. 1. Further comparative and structural studies are required.

Squamocin-K (**11**), a white wax, has the same molecular formula as **9** and **10**. The EI-MS of **11** was again similar to those of **9** and **10**. However, the $^1$H- and $^{13}$C-NMR spectra were different, exhibiting a characteristic *threo–trans–threo–trans–threo* pattern for the tetrahydrofuran portion. This was confirmed by the $^1$H-NMR spectrum of the diacetate of **11** (Table III). The $^1$H-NMR data for the di-($R$)-MTPA ester of **11** [$\delta$ 3.93 (H-17, -18), 4.00 (H-14, -21), 5.03 (H-13, -22)] allowed us to assign 13$R$,14$R$,17$R$,18$R$,21$R$,22$R$ configuration. Thus, squamocin-K was formulated as shown in **11**. This is a novel acetogenin.

Squamocins-L (**12**), -M (**13**) and -N (**14**) were $C_{37}$ bis-tetrahydrofuranic acetogenins, having the same molecular formula, $C_{37}H_{66}O_6$ [FAB-MS, $m/z$ 607 ($MH^+$)]. Their mass fragment patterns resembled one another, indicating a stereoisomeric relationship of their structures.

Squamocin-L (**12**), white crystals, mp 67.5—69 °C, exhibited $^1$H- and $^{13}$C-NMR spectra similar to those of **9** (or **10**). The EI-MS of **12** (Fig. 10) exhibited series of ion peaks starting from $m/z$ 295 (C-15/C-16 fission), 365 (C-19/C-20 fission) and 435 (C-23/C-24 fission). *Threo–trans–threo–trans–erythro* structure[17] of the bis-tetrahydrofuran portion was deduced from the $^{13}$C-NMR data of **12** (compare with the data for **7**). The $^1$H-NMR data for the ($R$)-MTPA ester of **12** [$\delta$ 3.64/3.83 (H-19 and -20), 3.94/5.24 (H-23/H-24), 4.00/5.02 (H-16/H-15)] allowed us to assign 15$R$,16$R$,19$R$,20$R$,23$R$,24$R$ configuration for **12**. These spectral data established the structure of squamocin-L to be as shown in **12**. Squamocin-L was identical with desacetyluvaricin, mp 63—65 °C, isolated from *U. accuminata*[6] as well as *A. glabra* seeds[26] on the basis of spectral and melting point comparison. This is the first report of isolation of **12** from *A. squamosa*.

Squamocin-M (**13**) was isolated as a colorless oil which solidified in a refrigerator. The bis-tetrahydrofuran portion of **13** appeared to have a symmetrical structure as evidenced by the $^1$H- and $^{13}$C-NMR spectra. The $^{13}$C-NMR data for **13** further showed *threo–trans–threo–trans–threo* stereochemistry for the bis-tetrahydrofuran portion (compare with, *e.g.*, **8** and **11**). The 15$R$,16$R$,19$R$,20$R$,23$R$,24$R$ configuration was assigned on the basis of the $^1$H-NMR data for the di-($R$)-MTPA ester [$\delta$ 3.93 (H-19, -20), 3.99 (H-16, -23) and 5.01 (H-15, -24)]. These spectral data established the structure of squamocin-M to be as shown in **13**. We consider that squamocin-M is identical with isodesacetyluvaricin[28] (mp below 30 °C),

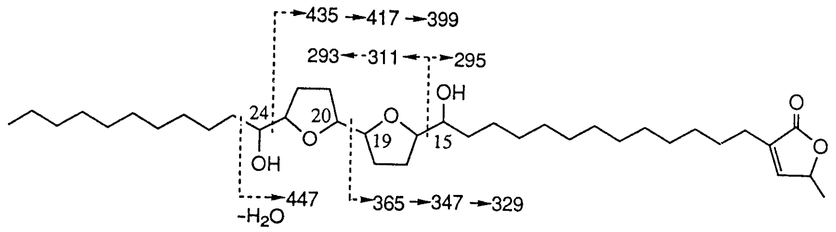

Fig. 10. Mass Fragmentation of Squamocin-L (**12**)

isolated from *U. narum*, on the basis of spectral comparison. This is the first report of isolation of **12** from *A. squamosa*.

Squamocin-N (**14**) was isolated as a colorless oil which solidified in a refrigerator. The $^1$H- and $^{13}$C-NMR data for **14** indicated that the bis-tetrahydrofuran portion is symmetrical, because the signals due to this portion of the molecule were duplicated. The C-15/C-16-*threo* and C-23/C-24-*threo* structure was also shown by the NMR data [$\delta_H$ 3.40 (H-15/23) and $\delta_C$ 74.0 (C-15/23)]. It can be seen from Table II that the $^{13}$C-NMR data for this portion were apparently different from those for the *threo–trans–threo–trans–threo* isomer (compare with, *e.g.*, **13**). *Threo–cis–threo–cis–threo*, *threo–cis–erythro–cis–threo*, and *threo–trans–erythro–trans–threo* structures can be considered as possible structures for **14**. These structures can be readily differentiated by analysis of the $^1$H-NMR data for the diacetate of **14**. As can be seen from Table III, the data for the *threo–cis–threo–cis–threo* structure were in good agreement with those for the diacetate of **14**. The absolute configuration of the tetrahydrofuran portion was investigated by applying the advanced Mosher method.[18] The $^1$H-NMR data for the (*R*)- and (*S*)-MTPA esters are listed in Table V. The sign of $\Delta_{S-R}$ for H-14/25, H-15/24 and H-19/20 was in accord with that predicted for 15*R*/24*R* configuration. Although a positive small $\Delta_{S-R}$ value of H-16/23 cannot be rationalized from the 15*R*/24*R* configuration, anomalous behavior of H-16/23 was previously noted.[15] On the basis of the above consideration, the structure of squamocin-N was established to be as shown in **14**. Compound **14** is the first example with a *threo–cis–threo–cis–threo* relationship.

In conclusion, we have carried out detailed chemical studies on thirteen adjacent bis-tetrahydrofuranic acetogenins isolated from *A. squamosa* and established their structures including absolute stereochemistry. The results of another approach using precursor-ion scanning mass spectrometry combined with the derivatization with *N*,*N*-dimethylethylenediamine for **2**—**14** corroborated the assigned structures.[16] Among these acetogenins, squamocins-D (**4**), -E (**5**), -F (**6**), -I (**9**), -K (**11**) and -N (**14**) are novel.

The structural features of the acetogenins of *A. squamosa* can be summarized as follows. 1) The number of carbon atoms in the molecule is either $C_{37}$ or $C_{35}$. 2) The position of hydroxyl substitution is C-4, C-12, C-28 (C-26 for $C_{35}$-acetogenins), and C-29. 3) Three types of structures regarding the relative stereochemistry of the adjacent bis-tetrahydrofuran portion are encountered. 4) The C-4 configuration is consistently *R* and the C-36 configuration is supposed to be *S*.

There are still another ten or so acetogenins which are difficult to obtain in a pure form even by repeated HPLC. Among these we have recently isolated a double bond-containing acetogenin, which is of great interest from a biosynthetic point of view.[29]

## Experimental

Melting points were determined on a Yazawa BY-1 hot-stage microscope and are uncorrected. $^1$H- and $^{13}$C-NMR spectra were recorded on a JEOL GSX-500 (500 MHz for $^1$H-NMR and 125 MHz for $^{13}$C-NMR) spectrometer in $CDCl_3$ solution with tetramethylsilane as an internal reference. IR spectra were determined on a JASCO IR-810 spectrometer in $CHCl_3$ solution. UV spectra were obtained on a Shimadzu UV-200 spectrometer in MeOH solution. CD spectra were measured with a JASCO J-500C polarimeter at 25 °C in MeOH solution. Optical rotations were measured with a JASCO DIP-360 polarimeter at 25 °C in MeOH solution. EI-(70 eV) and FAB-MS were obtained with a JEOL JMS-AX505HA spectrometer. *m*-Nitrobenzyl alcohol was used as the matrix for the measurement of FAB-MS. Precursor-ion spectrum (FAB) 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 Kieselgel 60 (70—230 mesh, 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 (15 cm × 6 mm i.d.) and an STR Prep-ODS column (25 cm × 20 mm i.d.) were used for analytical and preparative purposes, respectively.

**Isolation of Acetogenins** Pulverized seeds (1 kg) of *A. squamosa*, purchased at Varanasi, India were extracted with petroleum ether (bp 60—80 °C) using a Soxhlet continuous extractor. The extract was allowed to stand at room temperature for 24 h and the supernatant solution was separated. The oily residue was washed with petroleum ether and the washings were combined with the above supernatant. This was partitioned with MeOH–water (10 : 1) and the separated lower methanolic layer was concentrated. The residue was digested with AcOEt and the soluble part was concentrated to give an oil (8.1 g). This was chromatographed on a silica gel (250 g) column. Elution with a $CHCl_3$–AcOEt gradient system (2 : 1 to AcOEt alone) gave the least polar fraction. Continued elution of the column with an AcOEt–MeOH gradient system (AcOEt alone to 100 : 3) afforded the moderately polar fraction, and further elution with AcOEt–MeOH (100 : 3 to 100 : 10) afforded the polar fraction. The least polar fraction was further chromatographed on silica gel. Elution with benzene–AcOEt (5 : 1 to 3 : 1) removed non-polar lipid and further elution with benzene–AcOEt (3 : 1—AcOEt alone) afforded the non-polar fraction.

The above three fractions were then separated by preparative ODS-HPLC with MeOH–water (8 : 1, 10 : 1 and 11 : 1 for the polar, moderately polar and non-polar fractions, respectively; flow rate, 8 ml/min). When the separated acetogenin was slightly impure, it was purified by p-TLC and/or ODS-HPLC (solvent, $CH_3CN$–$H_2O$, appropriate combination such as 15 : 1, other conditions were essentially the same as described above). The amounts of the isolated acetogenins ranged from 70 mg of squamocin-K to 10 mg of squamocin-J.

**Determination of the C-36 Stereochemistry of Squamocin** DIBAH (0.5 ml) was added to a solution of squamocin (25 mg) in tetrahydrofuran (THF) (1 ml) under nitrogen at −78 °C and the mixture was stirred for 1 h at the same temperature. Extractive (ether) work-up afforded a crude product, which was dissolved in dry THF (2 ml) and stirred, after the addition of $LiAlH_4$ (15 mg), for 30 min at 0 °C. Extractive (ether) work-up gave a crude product, which was treated with dry pyridine (0.5 ml) and (*S*)-(+)-MTPA chloride (0.2 ml) at room temperature. Extractive (AcOEt) work-up gave a crude MTPA ester. The ester in $CH_2Cl_2$ (3 ml) was ozonized at −78 °C for 30 min and quenched by the addition of $Me_2S$ (50 $\mu$l). Extractive ($CH_2Cl_2$) work-up gave a crude aldehyde, which was dissolved in acetone (1 ml). The solution was stirred for 20 min at room temperature after addition of Jones reagent (0.1 ml). Extractive ($CH_2Cl_2$) work-up gave a crude product. This was treated with ethereal diazomethane. The crude ester was analyzed by TLC in comparison with authentic (2*R*)- and (2*S*)-methyl lactate (*R*)-MTPA esters. A spot was detected at the porition with $Rf=0.6$, where the authentic (*S*)-isomer appeared, but not at $Rf=0.7$, where the authentic (*R*)-isomer appeared (developed twice with hexane–AcOEt 7:1 on a Merck 5715 silica gel plate). A sample obtained by p-TLC from the band with $Rf=0.6$—0.7 was analyzed by GLC [a Shimadzu GC-MS DF 9020 spectrometer, Shimadzu capillary column HiCap CBP1-S25-050, 15 m, column temperature 130 °C, retention times for (*R*)- and (*S*)-samples were 9.1 and 9.8 min, respectively]. A peak was observed at 9.8 min, but not at 9.1 min.

**Preparation of Acetate Derivative** *Ca.* 2 mg of acetogenin was dissolved in pyridine (20 $\mu$l) and acetic anhydride (20 $\mu$l) and the solution was allowed to stand at room temperature overnight. MeOH (20 $\mu$l) was added and the solvent was removed by flushing with nitrogen. Separation of the product by p-TLC on silica gel afforded a purified acetate as an oil.

**Preparation of (*R*)-MTPA Ester** The ester was prepared according to a slight modification of the published method.[18)] Pyridine (20 $\mu$l) and (*S*)-(+)-MTPA chloride (8 $\mu$l) were added to ca. 2 mg of acetogenin. Precipitation of the hydrochloride salt occurred immediately. After ca. 0.5—1 h (completion of the reaction was confirmed by TLC), [3-(dimethyl)amino]propylamine (5 $\mu$l) was added. After a few minutes the mixture was diluted with AcOEt. Separation of the mixture by p-TLC furnished the purified (*R*)-MTPA ester. The (*S*)-MTPA ester was similarly prepared using (*R*)-(−)-MTPA chloride.

**Squamocin-B (2)** A white wax. $[\alpha]_D^{25}$ +27.6° ($c=0.2$, MeOH). IR $\nu_{max}$ cm$^{-1}$: 3660, 3575, 3450, 1745. HR-FAB-MS Calcd for $C_{35}H_{63}O_7$ ($MH^+$; $m/z$): 595.4574. Found: 595.4630. $^1H$-NMR $\delta$: 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).

Tri-(*R*)-MTPA ester, $^1H$-NMR $\delta$: 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), 3.515, 3.532, 3.609 (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).

**Squamocin-C (3)** White crystals, mp 50—51 °C. $[\alpha]_D^{25}$ +19.5° ($c=0.92$, MeOH). IR $\nu_{max}$ cm$^{-1}$: 3690, 3585, 3460, 1750. UV $\lambda_{max}$ nm ($\varepsilon$): 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 $\delta$: 0.88 (3H, t, $J=6.7$ Hz, H-34), 1.41 (3H, d, $J=7.1$ Hz, H-37), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.39 (1H, m, H-15), 3.58 (1H, m, H-29), 3.82—3.96 (5H, m, H-16, -19, -20, -23, -24), 4.99 (1H, q, $J=6.9$ Hz, H-36), 6.98 (1H, s, H-35).

Triacetate, FAB-MS of acetate $m/z$: 749 ($MH^+$). $^1H$-NMR $\delta$: 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).

Tri-(*R*)-MTPA ester, $^1H$-NMR $\delta$: 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), 3.66, 3.83 (1H each, m, H-19, -20), 3.90 (1H, m, H-23), 3.99 (1H, q, $J=7.3$ Hz, H-16), 4.99 (1H, qq, $J=6.6$, 1.5 Hz, H-36), 5.04 (2H, m, H-15, -29), 5.16 (1H, m, H-24), 6.98 (1H, s, H-35), 7.35—7.65 (15H, m, aromatic).

**Squamocin-D (4)** A colorless oil. $[\alpha]_D^{25}$ +30.1° ($c=0.58$, MeOH). IR $\nu_{max}$ 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 $\delta$: 0.88 (3H, t, $J=7.1$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), *ca.* 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), 6.99 (1H, s, H-35).

Triacetate, $^1H$-NMR $\delta$: 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, 2 × 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), 6.98 (1H, br s, H-35).

Tri-(*R*)-MTPA ester, $^1H$-NMR $\delta$: 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, MeO), 3.587 (3H, s, MeO), 3.599 (3H, s, MeO), 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, d, $J=1.5$ Hz, H-35), 7.35—7.64 (15H, m, aromatic).

**Squamocin-E (5)** White crystals, mp 48—50 °C (evaporated from $CHCl_3$). $[\alpha]_D^{25}$ +20.9° ($c=0.25$, MeOH). IR $\nu_{max}$ cm$^{-1}$: 3665, 3570, 3450, 1745. HR-FAB-MS Calcd for $C_{35}H_{63}O_7$ ($MH^+$; $m/z$): 595.4574. Found: 595.4592. $^1H$-NMR $\delta$: 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).

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

Tri-(*R*)-MTPA ester, $^1H$-NMR $\delta$: 0.88 (3H, t, $J=7.0$ Hz, H-32), 1.31 (3H, d, $J=7.1$ Hz, H-35), 2.60 (1H, br d, $J=15.6$ Hz, H-3a), 2.67 (1H, dd, $J=15.6$, 7.8 Hz, H-3b), 3.499, 3.598, 3.605 (3H each, s, MeO), 3.93 (2H, t, $J=6.5$ Hz, H-17, -18), 4.00 (2H, q, $J=7.0$ Hz, H-14, -21), 4.90 (1H, m, H-34), 5.03 (2H, m, H-13, -22), 5.37 (1H, m, H-4), 6.97 (1H, br s, H-33), 7.33—7.64 (15H, m, aromatic).

**Squamocin-F (6)** A white wax. $[\alpha]_D^{25}$ +21.0° ($c=0.58$, MeOH). IR $\nu_{max}$ cm$^{-1}$: 3560, 3450, 1750. HR-FAB-MS Calcd for $C_{37}H_{67}O_7$ ($MH^+$; $m/z$): 623.4887. Found: 623.4890. $^1H$-NMR $\delta$: 0.88 (3H, t, $J=7.1$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), 2.26 (2H, t, $J=7.7$ Hz, H-3), 3.40 (1H, m, H-24), 3.44 (1H, m, H-15), 3.60 (1H, m, H-12), 3.80—3.94 (4H, m, H-16, -19, -20, -23), 4.99 (1H, q, $J=7.1$ Hz, H-36), 6.99 (1H, s, H-35).

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

Tri-(*R*)-MTPA ester, $^1H$-NMR $\delta$: 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, MeO), 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 (7)** White crystals, mp 77—78 °C (from MeOH–water). $[\alpha]_D^{24}$ +28.5° ($c=0.50$, MeOH). IR $\nu_{max}$ cm$^{-1}$: 3580, 3450, 1750. CD (MeOH) $\Delta\varepsilon$ (nm): −0.40 (240). *Anal.* Found: C, 71.64; H, 10.64. Calcd for $C_{37}H_{66}O_7$: C, 71.34; H, 10.68. $^1H$-NMR $\delta$: 0.89 (3H, t, $J=5.9$ Hz, H-34), 1.41 (3H, d, $J=6.7$ Hz, H-37), 2.40 (1H, dd, $J=15.0$, 8.2 Hz, H-3a), 2.52 (1H, br d, $J=15.0$ Hz, H-3b), 3.38 (1H, m, H-15), 3.77—3.80 (3H, m, H-16, -23, -24), 3.80 (1H, m, H-4), 3.92 (2H, H-19, 20), 4.98 (1H, qq, $J=6.8$, 1.4 Hz, H-36), 6.98 (1H, br s, H-35).

Tris-(*R*)-MTPA ester, $^1H$-NMR $\delta$: 0.88 (3H, t, $J=7.1$ Hz), 1.31 (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, 3.543, 3.612 (3H each, s, OMe), 3.65 (1H, m, H-19 or -20), 3.83 (1H, m, H-20 or -19), 3.93 (1H, m, H-23), 4.00 (1H, q, $J=7.4$ Hz, H-16), 4.90 (1H, qq, $J=6.9$, 1.8 Hz, H-36), 5.03 (1H, q, $J=6.7$ Hz, H-15), 5.23 (1H, q, $J=5.9$ Hz, H-24), 5.38 (1H, m, H-4), 6.97 (1H, d, $J=1.5$ Hz, H-35), 7.34—7.66 (15H, m, aromatic).

Tri-(*S*)-MTPA ester, $^1H$-NMR $\delta$: 0.88 (3H, t, $J=6.7$ Hz), 1.28 (3H, d, $J=7.2$ Hz), 2.58 (2H, m, H-3), 3.519, 3.523, 3.549 (3H each, s, OMe), 3.79 (2H, m, H-19, -20), 3.99 (1H, m, H-23), 4.03 (1H, q, $J=8.7$ Hz, H-16), 4.86 (1H, q, $J=6.8$ Hz, H-36), 5.06 (1H, q, $J=6.4$ Hz, H-15), 5.25 (1H, m, H-24), 5.32 (1H, m, $J=5.7$ Hz, H-4), 6.73 (1H, d, $J=1.5$ Hz, H-35), 7.34—7.67 (15H, m, aromatic).

**Squamocin-H (8)** White crystals, mp 45—48 °C (from MeOH–$H_2O$). $[\alpha]_D^{24}$ +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 $\delta$: 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).

**Squamocin-I (9)** White needles, mp 68.5—71 °C (from MeOH–$H_2O$). $[\alpha]_D^{24}$ +22.2° ($c=0.50$, MeOH). IR $\nu_{max}$ cm$^{-1}$: 3560, 3450, 1750.

UV $\lambda_{max}$ nm (log $\varepsilon$): 209 (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. $^1$H-NMR $\delta$: 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, $J$=7.0 Hz, H-34), 6.99 (1H, br s, H-33).

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, AcO), 2.074 (3H, s, AcO), 2.26 (2H, t, $J$=7.7 Hz, H-3), 3.89 (2H, m, H-17, -18), 3.98 (2H, qui, 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, $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=7.0 Hz, H-32), 1.40 (3H, d, $J$=7.0 Hz, H-35), 2.26 (2H, t, $J$=7.7 Hz, H-3), 3.543, 3.611 (3H each, s, MeO), 3.65, 3.83 (1H each, m, H-17, 18), 3.93 (1H, m, H-14), 4.00 (1H, q, $J$=7.0 Hz, H-21), 4.99 (1H, q, $J$=7.0 Hz, H-34), 5.03 (1H, q, $J$=7.8 Hz, H-22), 5.23 (1H, q, $J$=6.0 Hz, H-13), 6.98 (1H, d, $J$=1.5 Hz, H-33), 7.34—7.65 (10H, m, aromatic).

**Squamocin-J (10)** White needles, mp 85-86.5 °C (from MeOH–$H_2O$). $[\alpha]_D^{25}$ +18.6° ($c$=0.42, MeOH). IR $\nu_{max}$ cm$^{-1}$: 3560, 3450, 1750. UV $\lambda_{max}$ nm (log $\varepsilon$): 209 (3.8). CD (MeOH) $\Delta\varepsilon$(nm): −0.40 (241). *Anal.* Found: C, 73.08; H, 10.72. Calcd for $C_{35}H_{62}O_6$: C, 72.62; H, 10.80. $^1$H-NMR $\delta$: 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-13), 3.82—3.90 (3H, m, H-14, -21, -22), 3.90—3.97 (2H, m, H-17, -18), 5.00 (1H, q, $J$=7.0 Hz, H-34), 6.99 (1H, br s, H-33).

Diacetate, $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=6.9 Hz, H-32), 1.41 (3H, d, $J$=6.6 Hz, H-35), 2.047 (3H, s, AcO), 2.074 (3H, s, AcO), 2.26 (2H, t, $J$=7.7 Hz, H-3), 3.89 (2H, m, H-17, -18), 3.98 (2H, qui, 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, $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=7.3 Hz, H-32), 1.40 (3H, d, $J$=6.5 Hz, H-35), 2.26 (2H, t, $J$=7.7 Hz, H-3), 3.543, 3.621 (3H each, s, OMe), 3.65, 3.83 (1H each, m, H-17, -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 (11)** A white wax, $[\alpha]_D^{25}$ +20.5° ($c$=0.53, MeOH); IR $\nu_{max}$ cm$^{-1}$, 3560, 3450, 1750; *Anal.* Found: C, 72.34; H, 10.96. Calcd for $C_{35}H_{62}O_6$: C, 72.62; H, 10.80. $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=6.9 Hz, H-32), 1.40 (3H, d, $J$=6.7 Hz, H-35), 2.26 (2H, t, $J$=8.0 Hz, H-3), 3.39 (2H, m, H-13, -22), 3.81—3.87 (2H, q, $J$=6.8 Hz, H-14, -21), 3.87—3.93 (2H, m, H-17, -18), 4.99 (1H, q, $J$=6.8 Hz, H-34), 6.98 (1H, d, $J$=1.4 Hz, H-33).

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

Di-(*R*)-MTPA ester, $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=7.0 Hz, H-32), 1.40 (3H, d, $J$=7.0 Hz, H-35), 2.26 (2H, t, $J$=8.0 Hz, H-3), 3.603, 3.606 (3H each, s, MeO), 3.93 (2H, t, $J$=6.5 Hz, H-17, -18), 4.00 (2H, q, $J$=7.0 Hz, H-14, -21), 4.99 (1H, q, $J$=6.8 Hz, H-34), 5.03 (2H, 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 (12)** White crystals, mp 67.5—69 °C (from MeOH–$H_2O$). $[\alpha]_D^{25}$ +19.3° ($c$=0.98, MeOH). IR $\nu_{max}$ cm$^{-1}$: 3590, 3450, 1745. *Anal.* Found: C, 73.01; H, 11.25. Calcd for $C_{37}H_{66}O_6$: C, 73.22; H, 10.96. $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=6.9 Hz, H-34), 1.41 (3H, d, $J$=6.7 Hz, H-37), 2.26 (2H, t, $J$=6.7 Hz, H-3), 3.40 (1H, m, H-15), 3.81—3.90 (3H, m, H-16, -23, -24), 3.91—3.98 (2H, m, H-19, -20), 5.00 (1H, q, $J$=7.0 Hz, H-36), 6.99 (1H, br s, H-35).

Di-(*R*)-MTPA ester, $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=6.8 Hz, H-34), 1.41 (3H, d, $J$=6.8 Hz, H-37), 2.27 (2H, t, $J$=7.6 Hz, H-3), 3.545 (3H, s, OMe), 3.617 (3H, s, OMe), 3.64, 3.83 (1H each, m, H-19, -20), 3.94 (1H, m, H-23), 4.00 (1H, q, $J$=6.8 Hz, H-16), 5.02 (2H, m, H-15, -36), 5.24 (1H, q, $J$=6.0 Hz, H-24), 6.98 (1H, s, H-35), 7.35—7.68 (10H, m, aromatic)

**Squamocin-M (13)** A colorless oil. $[\alpha]_D^{25}$ +26.0° ($c$=0.55, MeOH). *Anal.* Found: C, 73.01; H, 11.25. Calcd for $C_{37}H_{66}O_6$: C, 73.22; H, 10.96. $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=6.9 Hz, H-34), 1.40 (3H, d, $J$=6.9 Hz, H-37), 2.26 (2H, t, $J$=7.7 Hz, H-3), 3.39 (2H, m, H-15, -24), 3.81—3.87 (2H, q, $J$=6.0 Hz, H-16, -23), 3.87—3.93 (2H, m, H-19, -20), 4.99 (1H, q, $J$=6.8 Hz, H-36), 6.98 (1H, d, $J$=1.4 Hz, H-35).

Di-(*R*)-MTPA ester, $^1$H-NMR $\delta$: 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 Hz, H-3), 3.606 (6H, s, OMe), 3.93 (2H, t, $J$=6.1 Hz, 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 (10H, m, aromatic)

**Squamocin-N (14)** A colorless oil. $[\alpha]_D^{25}$ +40.6° ($c$=0.43, MeOH). IR: $\nu_{max}$ cm$^{-1}$: 3560, 3450, 1750. CD (MeOH) $\Delta\varepsilon$(nm): −0.57 (240). *Anal.* Found: C, 73.01; H, 11.25. Calcd for $C_{37}H_{66}O_6$. C, 73.22; H, 10.96. $^1$H-NMR $\delta$: 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—3.86 (2H, q, $J$=5.8 Hz, H-16, -23), 3.88—3.93 (2H, m, H-19, -20), 4.99 (1H, q, $J$=6.8 Hz, H-36), 6.98 (1H, d, $J$=1.3 Hz, H-35).

Diacetate, $^1$H-NMR $\delta$: 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, $^1$H-NMR $\delta$: 0.88 (3H, t, $J$=6.8 Hz, H-34), 1.40 (3H, d, $J$=6.8 Hz, H-37), 2.26 (2H, t, $J$=8.1 Hz, H-3), 3.648, (6H, s, MeO), 3.90 (4H, m, 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, $^1$H-NMR $\delta$: 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, qui, $J$=5.0 Hz, H-19, -20), 3.95 (2H, q, $J$=6.5 Hz, H-16, -23), 4.99 (1H, q, $J$=6.6 Hz, H-36), 5.16 (2H, q, $J$=6.3 Hz, H-15, -24), 6.98 (1H, br s, H-35), 7.35—7.65 (10H, m, aromatic).

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