Squamosten-A, a Novel Mono-tetrahydrofuranic Acetogenin with a Double Bond in the Hydrocarbon Chain, from *Annona squamosa* L.

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Squamosten-A, a new mono-tetrahydrofuranic acetogenin possessing a double bond in the hydrocarbon chain, has been isolated from the seeds of *Annona squamosa* L. (Annonaceae). The structure has been elucidated on the basis of spectral evidence, including precursor-ion scanning spectra. Chemical degradation was successfully employed to determine the position of the double bond.

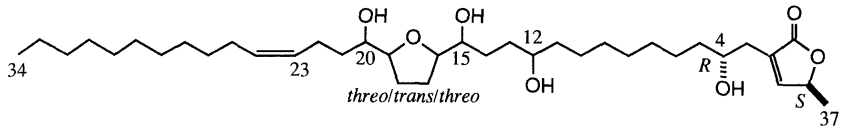
Keywords squamosten-A; tetrahydrofuranic acetogenin; *Annona squamosa*; Annonaceae; precursor-ion scanning

A group of tetrahydrofuranic acetogenins have recently been isolated from limited genera of Annonaceae plants.[1] These acetogenins exhibit various biological activities, *viz.*, 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] Our earlier study on the seeds of *A. squamosa* led to the isolation of squamocin[3] and squamostatin-A,[4] and subsequent study has resulted in the isolation of more than fifteen bis-tetrahydrofuranic acetogenins of this group.[5] Our further study has yielded yet another tetrahydrofuranic acetogenin, named squamosten-A (**1**). Squamosten-A has a new structural feature that a double bond is embedded in the hydrocarbon chain. In this paper we report the structure elucidation of this novel acetogenin.

Petroleum ether extract of the seeds of *A. squamosa* was processed as described earlier.[3] The partially purified acetogenin fraction, which has a polarity between those of squamocin and squamostatin-A on silica gel TLC, was further purified by reversed-phase HPLC to give squamosten-A (**1**).

Squamosten-A (**1**) was obtained as a white solid, mp 64—67 °C. The molecular formula, $C_{37}H_{66}O_7$, was established from the high resolution FAB-MS (HRFAB-MS) (MH^+ *m/z* 623) and ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR spectra (Table I) exhibited signals characteristic of tetrahydrofuranic acetogenins, *e.g.*, the signals of an α,β-unsaturated γ-lactone moiety; δ_H 1.39 (H-37), 5.05 (H-36), 7.18 (H-35); δ_C 174.58 (C-1), 131.24 (C-2), 151.78 (C-35), 77.96 (C-36), 19.12 (C-37) and the signals of a hydrocarbon chain with a terminal methyl group: δ_H 0.88; δ_C 14.10. The UV (210 nm) and IR (1745 cm^{-1}) spectral data also supported the presence of the γ-lactone moiety.

The FAB-MS of **1** suggested the presence of four hydroxyl groups in the molecule, showing ions arising from successive loss of water molecules from the molecular ion [*m/z* 605 ($MH^+ - H_2O$), 587, 569, 551]. The ^{1}H-NMR spectrum exhibited the signals of six oxymethine protons (H-36 is not counted). Among these, one hydrogen signals at δ 3.83 was readily assigned to H-4 because the C-3 methylene protons were observed as a characteristic ABX system,[6] and two other hydrogen signals at δ 3.83 were assigned to those adjacent to the tetrahydrofuran ring (H-16, H-19). Another two hydrogen signals at δ 3.44, which are coupled to the signals at δ 3.83, were assigned to H-15 and H-20. The last oxymethine hydrogen signal, which is coupled only to hydrocarbon methylene protons, was ascribable to the oxymethine hydrogen in the middle of the hydrocarbon chain. Notably, two olefinic protons, coupled to each other with $J = 11.0$ Hz (the J value was measured by selective decoupling of H_2-22 and -25), were also discerned in the ^{1}H-NMR spectrum at δ 5.36 and 5.39 (m, each). The ^{13}C-NMR spectrum was also in agreement with the above assignments, exhibiting six oxymethine carbons at δ 69.99, 71.70, 73.50, 74.37, 82.63, 83.65 (C-36 is not counted). Among these, the two downfield signals (δ 82.63, 83.65) were apparently ascribable to the tetrahydrofuran C-2 and C-5 carbons (C-16, C-19). The signals at δ 69.99 could be assignable to C-4 according to the literature value.[6] The signal at δ 71.70 had a value typical of an oxymethine carbon in the middle of a hydrocarbon chain. The remaining oxymethine signals (δ 74.37, 73.50) must be assigned to the carbons (C-15, C-20) adjacent to the tetrahydrofuran ring. Two olefinic carbon signals were also seen at δ 128.92 and 130.87. On the basis of the spectral data presented, squamosten-A is considered to be a new acetogenin containing one double bond and one hydroxyl group somewhere in the hydrocarbon chain.



squamosten-A (**1**)

The electron impact-MS (EI-MS) of **1** displayed a base-peak ion at *m/z* 309 and dehydrated ions thereof (Fig. 1). Another series of fragment ions starting with *m/z* 379 was also displayed. Since in the MS of these acetogenins fragment ion peaks arising from glycol fission are clearly observed, the tetrahydrofuran was able to be positioned through C-16 to C-19. However, the *m/z* 309 ion can be explained in two ways. One is that the ion is formed by the cleavage at C-15/C-16 of a structure bearing a double bond somewhere between C-5 and C-14 in the hydrocarbon chain. The other is that the ion is produced by the elimination of water from the structure initially formed by C-15/C-16 cleavage. We employed a precursor-ion scanning technique[7] to distinguish the two possibilities. Thus, precursor ions which afford *m/z* 309 as the product ion were scanned. As can be seen in Fig. 2, a significant ion peak was detected at *m/z* 327, clearly indicating that the *m/z* 309 ion is produced from the *m/z* 327 ion by the elimination of water. Similarly, the *m/z* 379 ion was found to be derived from the *m/z* 397 ion by the loss of water. It is now evident that the unsettled hydroxyl group must be positioned in the methylene chain between C-5 and C-14, whereas the double bond must be placed somewhere in the methylene chain beyond C-21.

TABLE I. NMR Data of Squamosten-A (**1**)

Atom	δ_H	δ_C
1	—	174.58
2	—	131.24
3	2.53 (br d, 14.6)	33.38
	2.40 (dd, 14.6, 8.3)	
4	3.83 (m)	69.99
5	*ca.* 1.5 (m)	37.39
6	*a)*	25.49*c)*
7—9	*a)*	*b)*
10	*a)*	25.61*c)*
11	1.5—1.7 (m)	37.54
12	3.63 (m)	71.70
13	1.5—1.7 (m)	33.59*d)*
14	*ca.* 1.5 (m)	*b)*
15	3.44 (m)	74.37*e)*
16	3.83 (m)	82.63*f)*
17	*ca.* 1.7, 2.0 (m)	28.74*g)*
18	*ca.* 1.7, 2.0 (m)	28.71*g)*
19	3.83 (m)	83.65*f)*
20	3.44 (m)	73.50*e)*
21	*ca.* 1.7 (m)	33.55*d)*
22	2.17 (m)	23.33
23	5.36 (m)	128.92
24	5.39 (m)	130.87
25	2.04 (q, 7.6)	27.25
26—31	*a)*	*b)*
32	*a)*	31.93
33	*a)*	22.69
34	0.88 (t, 7.3)	14.10
35	7.18 (q, 1.8)	151.78
36	5.05 (qq, 7.3, 1.8)	77.96
37	1.39 (d, 7.3)	19.12

a) 1.2—1.4 (m). *b)* 29.35—29.74. *c—g)* Assignments indicated by a given letter may be interchanged.

The position of the hydroxyl group was determined by analysis of the EI-MS of **1**, which exhibited ion peaks at *m/z* 269 and 251. The two ions are considered to be formed by cleavage at C-12/C-13 (Fig. 2). Thus, the hydroxyl group was located at the C-12 position. This assignment was further supported by the analysis of the chemical shifts of the ^{13}C-NMR spectrum of **1**. The signal at δ *ca.* 33.6, was shifted upfield by *ca.* 4 ppm from the value expected for a carbon alpha to a hydroxyl bearing carbon (see the chemical shift of C-5 or C-11). This upfield shift can be reasonably explained in terms of the beta-effect of a hydroxyl group, provided that the unsettled hydroxyl group is placed either at C-7 or C-12. The aforementioned MS data are consistent only with the C-12 hydroxyl structure.

The position of the double bond was determined by a chemical method.[8] Treatment of **1** with $NaIO_4$ and $RuCl_3$[9] afforded degradation products, which were then converted into *p*-bromophenacyl esters. HPLC analysis of the esters showed a peak possessing the same retention time as that of an authentic sample of *p*-bromophenacyl undecanoate. Thus, the double bond was unambiguously determined to be at the C-23 position. The structure of squamosten-A was, therefore, established to be as shown in the structure **1**.

The stereochemical relationships at C-15/C-16 and C-19/C-20 were defined as *threo* on the basis of the chemical shifts of 15-H, 16-H, 19-H, 20-H as well as 15-C and 20-C according to the empirical rule.[1] *trans*-Relationship of the substituents on the tetrahydrofuran ring was obtained by ^{1}H-NMR comparison with model compounds.[10] The stereochemistry at C-36 was determined as *S* based on the negative sign of the Cotton effect at 240 nm.[5,11] The stereochemistry at C-4 was elucidated to be *R* on the basis of the ^{1}H-NMR data of the (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid

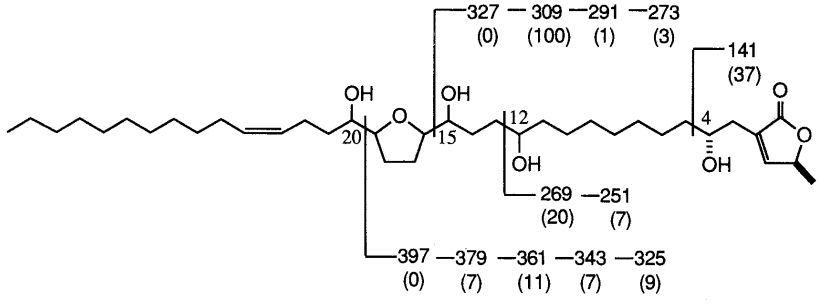


Fig. 1. Mass Fragmentation of Squamosten-A (**1**)

Numbers in parentheses indicate relative intensity (%) with respect to the ion peak at *m/z* 309.

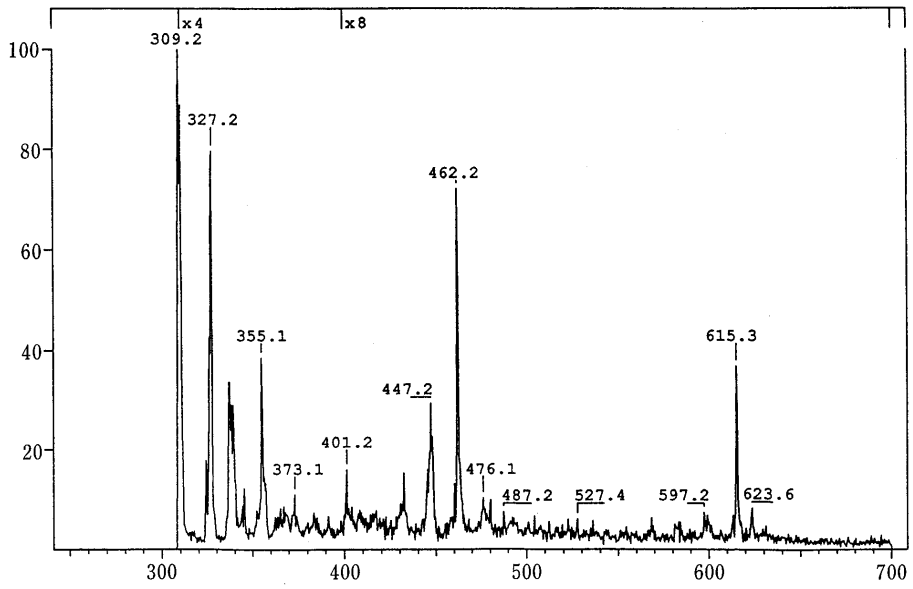


Fig. 2. Precursor Ion Scanning with the *m/z* 309 Ion as a Product Ion
The ions at *m/z* 462 and 615 correspond to 309 + *m*-nitrobenzyl alcohol and 309 + 2 × *m*-nitrobenzyl alcohol ions.

(MTPA) ester derivative of **1**. The chemical shifts of H-4 (δ 5.32), H-35 (δ 6.96), H-36 (δ 4.90) and H-37 (δ 1.31) were in good agreement with those of acetogenins with 4*R*,36*S* stereochemistry.[5,12]

The present isolation of squamosten-A (**1**) provides evidence to support a postulated polyketide biogenetic pathway. The location of the C-12 hydroxyl group is biosynthetically related with the C-12 oxygen functionality of squamostatin-A. Squamocin-type compounds would be produced *via* enzymatic epoxidation at the C-23 double bond and subsequent intramolecular cyclization to form a tetrahydrofuran ring at the C-20 to C-23 position with the C-24 hydroxyl group (the presence of C-4 or C-28 hydroxyl seems to be arbitrary).

Quite recently, a related mono-tetrahydrofuranic acetogenin with the double bond has been isolated from *Coniothalamus giganteus* (Annonaceae).[13]

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GSX-500 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. The IR spectrum was determined on a JASCO IR-810 spectrometer, and the UV spectrum on a Shimadzu UV-200 spectrometer. FAB and EI-MS were obtained with a JEOL JMS-AX505HA while precursor-ion scanning FAB-MS were obtained with a Finnigan MAT TSQ-700 spectrometer using *m*-nitrobenzyl alcohol as a matrix.

Isolation of Squamosten-A (1) The ground seeds of *A. squamosa* (2 kg), purchased at Varanasi, India, were extracted with petroleum ether (60—80 °C). The extract was concentrated and the thick waxy mass settling at the bottom was separated from the rest of the extract by decantation. The waxy semi-solid was washed with petroleum ether and chromatographed over silica gel. Elution of the column with solvent of increasing polarity furnished an oil from the EtOAc–MeOH (20 : 1) fraction (this fraction was eluted after squamocin, and contained some squamostatin-A). HPLC [Shimadzu STR-PREP-ODS 20 × 250 mm, MeOH–H_2O (20 : 1), CH_3CN–H_2O (20 : 1), 220 nm] separation of this oil afforded **1** (9 mg) as a white solid, mp 64—67 °C, $[\alpha]_D^{24}$ +9° (*c* = 0.1, MeOH). IR $\nu_{max}^{CHCl_3}$: 3665, 3575, 3450, 1745 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 210. (7000). CD (*c* = 0.067, MeOH) $\Delta\varepsilon^{25}$: −0.57 (240, negative maximum). *Rf* value: 0.48 (0.56 for squamocin and 0.44 for squamostatin-A) on a Merck precoated silica gel plate with AcOEt–$CHCl_3$–MeOH (10 : 4 : 1) as a developing solvent. HPLC t_R: 15.0 min (11.4 min for squamocin and 7.7 min for squamostatin-A) on a Shim-pack CLC ODS column (15 cm × 6 mm i.d.) with MeOH–H_2O 13 : 1 as an eluting solvent, flow rate 0.6 ml/min. HRFAB-MS (*m/z*): Calcd for $C_{37}H_{67}O_7$ (MH^+): 623.4887. Found: 623.4844.

About 1 mg of **1** was converted into the tetra-(*R*)-MTPA ester in a usual manner [(+)-(*S*)-MTPA chloride and pyridine]. ^{1}H-NMR δ: 0.88 (3H, t, *J* = 7.0 Hz), 1.31 (3H, d, *J* = 6.9 Hz), 2.60 (1H, br d, *J* = 13.0 Hz), 2.68 (1H, dd, *J* = 15.4, 7.7 Hz), 3.48, 3.50, 3.51, 3.53 (3H each, s), 3.93 (1H, q, *J* = 6.8 Hz), 4.02 (1H, m), 4.91 (2H, m), 4.98 (1H, m), 5.04 (1H, m), 5.22 (1H, m), 5.37 (2H, m), 6.96 (1H, s), 7.31—7.63 (20H, m).

Determination of the Position of the Double Bond A mixture of **1** (55 μg), $NaIO_4$ (100 μg) and $RuCl_3$ (catalytic amount) in H_2O, CH_3CN and CCl_4 (10 μl each) was stirred for 1 h at room temperature. The reaction mixture was partitioned between aqueous saturated $NaHCO_3$ and CCl_4. The separated aqueous layer was acidified with 2 N HCl, and then extracted with ether. The solvent was evaporated from the organic layer and the residue was mixed with *p*-bromophenacyl bromide (200 μg), CH_3CN (100 μl) and *N*,*N*-diisopropylethylamine (catalytic amount) and allowed to react for 2 h at room temperature. A portion of the reaction mixture was directly analyzed HPLC (column, TSK gel (ODS-120T, 25 cm × 3 mm); eluent, CH_3CN–H_2O (20 : 1); flow rate, 0.5 ml/min; UV detection at 254 nm). The HPLC showed a peak (13.3 min) corresponding to that of *p*-bromophenacyl undecanoate. Other reference esters had the following retention times: octanoate (9.1 min), nonanoate (10.1 min), decanoate (11.4 min) and dodecanoate (15.7 min). No peaks were detected at retention times other than that of undecanoate. The overall yield of *p*-bromophenacyl undecanoate was estimated as 45% on the basis of UV response (254 nm).

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10) The NMR data of **1** were compared with those of *trans*- and *cis*-2,5-di-(1-hydroxylpentyl)tetrahydrofurans (*threo*/*threo*-isomer). The chemical shifts of H-3/H-4 as well as C-3/C-4 of the tetrahydrofuran ring (δ 28.75 for the *trans*-isomer and δ 28.12 for the *cis*-isomer; compare with C-17/C-18 of **1**) can be used to differentiate *trans*/*cis*-structure. Details of the synthesis of the model compounds and spectral comparison will be reported elsewhere.
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