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Squamocin, a New Cytotoxic Bis-tetrahydrofuran Containing Acetogenin from *Annona squamosa*

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Squamocin, a new trihydroxy-bis-tetrahydrofuran fatty acid γ-lactone (acetogenin), has been isolated from *Annona squamosa* L. (Annonaceae) and its structure has been elucidated on the basis of spectral evidence and chemical degradations.

Keywords——squamocin; *Annona squamosa*; Annonaceae; acetogenin; bis-tetrahydrofuran; cytotoxic activity; α,β-unsaturated-γ-lactone; 2D-NMR

Annona squamosa L. (Annonaceae) is well known in India for its edible fruits. Seeds of the fruits are traditionally used as an abortificiant and are reported to have insecticidal properties.[2)] A preliminary screening of the petroleum ether extract of the seeds of *A. squamosa* showed substantial cytotoxic activity. Separation of the active component by column chromatography on silica gel led to the isolation of a new trihydroxy-bis-tetrahydrofuran fatty acid γ-lactone, which we named squamocin (**1**).

Squamocin was obtained as a solid which melts just above room temperature. The electron-impact mass (EI-MS) spectrum of **1** did not display a molecular ion peak but a series of peaks (*m/z* 604, 586 and 568) arising from loss of water were observed. The molecular weight was established as 622 from its fast atom bombardment mass (FAB-MS) spectrum and its molecular formula as $C_{37}H_{66}O_7$, which was corroborated by the microanalytical (C and H) data. The infrared (IR) spectrum of **1** showed bands characteristic of hydroxyl (3585 and 3460 cm^{-1}) and α,β-unsaturated γ-lactone (1755 cm^{-1}). The ultraviolet (UV) maximum at 215 nm also supported the presence of the latter functionality.

Spectral characteristics, including the proton and carbon-13 nuclear magnetic resonance

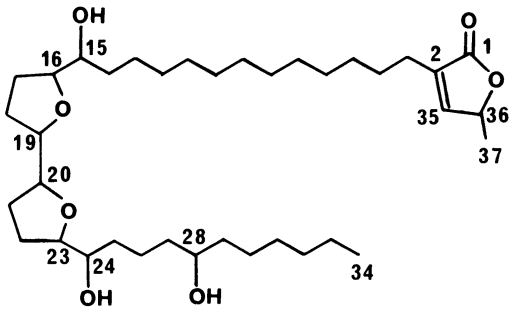

Fig. 1. Structure of Squamocin (**1**)

(^{1}H- and ^{13}C-NMR) data, suggested that squamocin (**1**) belongs to a growing family of bis-tetrahydrofuran-containing bioactive acetogenins,[3] which include uvaricin, rollinicin, isorollinicin, rollinone, desacetyluvaricin, cherimoline, dihydrocherimoline, 14-hydroxy-25-desoxyrollinicin, and, most recently, asimicin. These compounds characteristically contain 37 carbons, a γ-lactone moiety, two tetrahydrofuran rings and a few hydroxyl groups on a long hydrocarbon chain.

The presence of an α,β-unsaturated-γ-lactone moiety with an alkyl substituent at the α-position to the lactone carbonyl was easily confirmed from the ^{1}H-NMR spectrum (H-35, H-36 and H-37) and ^{13}C-NMR spectrum (C-1, -2, -35, -36 and -37). ^{1}H–^{1}H correlation spectroscopy (COSY) experiments clearly showed the connectivity of H-37 to H-36 (J=6.8 Hz), H-36 to H-35 (J=1.4 Hz), H-35 to H-3 (J=1.4 Hz, allylic coupling), and H-36 to H-3 (J=1.4 Hz, homo-allylic coupling). The H-3 methylene protons at δ2.21 (tt, J=7.7, 1.4 Hz) further coupled to methylene protons at δ 1.5 (H-4). An alkyl chain terminating with a methyl group was obvious from ^{1}H–^{1}H, ^{13}C–^{1}H and ^{13}C–^{1}H long-range COSY experiments, which revealed a connectivity of C-32/C-33/C-34 (H-34, δ0.88, unsymmetrical triplet).

In addition to H-36 (C-36), there are seven oxy-methine multiplet protons [δ 3.33 (1H), 3.52 (1H), 3.76 (3H), and 3.86 (2H)],[4] all of which were correlated to the oxygen-bearing methine carbons by ^{13}C–^{1}H COSY experiments. Four of these seven carbons, which appeared at relatively lower field (δ82.2—83.4), were assigned to carbons at the 2 and 5 positions of tetrahydrofuran rings and the remaining three appearing between δ 71.5—74.1 were obviously due to hydroxy-bearing carbons. The occurrence of three secondary hydroxyl groups in **1** was further confirmed by the following observations. The ^{1}H-NMR spectrum of **1**, after addition of trichloroacetyl isocyanate, a well known reagent for determining the number of hydroxyl groups,[5] exhibited three NHCO protons (δ8.47, 8.53, and 8.57) together with a significant downfield shift of the three protons at δ 3.33, 3.52 and 3.76 in **1** to the region of δ4.75—5.05 (CHOCONHCOCl$_3$) and a slight downfield shift of the protons at δ 3.76 (2H) to δ4.05 (H-16 and H-23).

Detailed analysis of the ^{1}H–^{1}H COSY spectrum of **1** revealed that H-15 (δ 3.33, dt J=11, 7.5 Hz; C-15, δ 74.1) is coupled to a proton at δ 3.76 (H-16). The ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra further indicated that the protons at δ 3.76 (presumably 2H) are coupled to methylene protons (on C-17 and C-22) at δ 1.5 and 1.9, and that the protons at δ 3.86 have a coupling with methylene protons (on C-18 and C-21) at δ 1.5 and 1.9/δ 1.76 and 1.87 (the δ 1.76 proton exhibits a coupling with the protons appearing at δ 1.87, 1.9, 1.5, and 3.86).[6] The four methylene units described above (carbon signals at δ28.96, 28.99, 28.48, and 24.97) are assumed to be parts of ring systems from the non-equivalence of each of the methylene protons, and this in turn supports the presence of two tetrahydrofuran rings. Difference in the chemical shifts of the protons and carbons adjacent to the bis-tetrahydrofuran rings, *e.g.* H-15 and H-24, is commonly encountered in this family of natural compounds due to non-symmetric stereochemical nature.[3,7] The spectral data mentioned above are consistent with the idea that **1** is a trihydroxy-bis-tetrahydrofuran fatty acid γ-lactone.

The only problem that remained to be solved was the location of the oxygen functionalities and this was settled by chemical means. Jones oxidation of **1** afforded a mixture of two acids (**2a** and **3a**). The major constituent **2a**, which was obtained in a pure form by silica gel column chromatography, was elucidated to be a C_{18}-acid, on the basis of the ^{1}H- and ^{13}C-NMR spectra (Table I) and MS [acid **2a**: FD-MS m/z 311 (M+H), EI-MS m/z 292 (M−H_2O); methyl ester **2b**: EI-MS m/z 306 (M−H_2O), 292 (M−MeOH)]. It is clear that **2a** corresponds to C-1—C-15/C-35—C-37 of **1**.

The minor constituent **3a** was characterized as 5-oxoundecanoic acid on the basis of the ^{13}C-NMR data of **3a** and the EI-MS of the methyl ester **3b**. Strong ion peaks at m/z 144 and 112 due to McLafferty rearrangement have a diagnostic value for the determination of the

TABLE I. ^{1}H- and ^{13}C-NMR Chemical Shifts (δ) and ^{1}H–^{1}H Coupling Constants (J, Hz) of Compounds **1**, **2a** and **3a**

Atom	**1**		**2a**		**3a**
	δ (^{13}C)	δ (^{1}H) (J)	δ (^{13}C)	δ (^{1}H) (J)	δ (^{13}C)
1	173.93 s	—	173.7	—	
2	134.31 s	—	134.3	—	
3	25.18 t	2.21 tt (7.7, 1.4)	25.2	2.26 t (7.1)	
4	27.43 t	1.5 m	27.5	1.58 qui (7.3)	
5	29.18 t	1.20—1.27 m	29.2[f]	1.20—1.35 m	
6	29.42[a] t		29.4[f]		
7	29.61[a] t		29.6[f]		
8	29.61[a] t		29.6[f]		
9	29.61[a] t		29.6[f]		
10	29.51[a] t		29.5[f]		
11	29.32[a] t		29.3[f]		
12	29.16[a] t		29.1[f]		
13	25.69 t		24.8	1.58 qui (7.3)	
14	33.12 t	1.3 m	34.0	2.33 t (7.1)	
15	74.09 d	3.33 dt (11, 7.5)	178.8		
16	83.36[b] d	3.76 m			
17	28.96[c] t	1.5, 1.9 m			
18	28.48[c] t	1.5, 1.9 m			
19	82.85[d] d	3.86 m			
20	82.55[d] d	3.86 m			
21	24.97[c] t	1.76, 1.87 m			
22	28.99[c] t	1.5, 1.9 m			
23	82.15[b] d	3.76 m			
24	71.51 d	3.76 m			179.0
25	32.49 t	1.3 m			32.9
26	22.07 t	1.3, 1.6 m			18.5
27	37.48[e] t	1.35—1.4 m			42.8[g]
28	71.60 d	3.52 m			210.5
29	37.23[e] t	1.35—1.4 m			41.2[g]
30	25.65 t	1.3 m			23.7
31	29.78 t	1.25 m			28.8
32	31.88 t	1.25 m			31.5
33	22.63 t	1.25 m			22.4
34	14.08 q	0.83 t (7.0)			13.9
35	148.97 d	6.96 q (1.4)	148.8	7.0 q (1.4)	
36	77.35 s	4.95 qq (6.8, 1.4)	77.4	5.0 qq (6.8, 1.4)	
37	19.21 q	1.36 d (6.8)	19.3	1.42 d (6.8)	

a—*g*) May be interchanged within the group [the signals at δ 24.97 t; 1.76, 1.87 m are ascribable to either C-18 or C-21 rather than C-17 or C-22].

position of the oxo functionality.[8] In addition, the carbon signal at δ 18.5 in **3a** can be rationalized in terms of the 5-oxo compound rather than any other ketone positional isomers. Finally, the structure of **3a** was confirmed by direct comparison of the ^{13}C-NMR spectrum with that of authentic **3a**[9] and by comparisons of the mass spectrum and retention time in gas chromatography (GC) of **3b** with those of authentic **3b**.[9]

Formation of the two fragment structures **2a** and **3a** can be interpreted by assuming oxidative cleavage of a glycol-type moiety present in **1**, since no carbonyl group other than that of the lactone moiety is present in **1**. It is clear that the ketone group of **3a** is an alcohol in **1**. Hence, the positions of the three hydroxyl groups were unambiguously established to be at

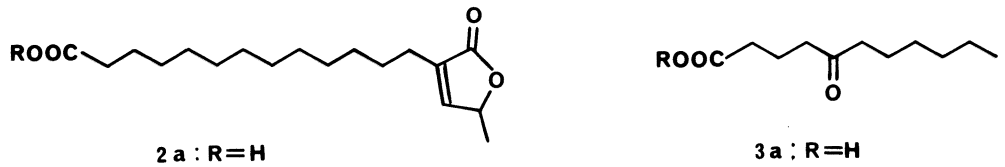

Fig. 2. Structure of Jones Oxidation Products **2a** and **3a**, and Their Methyl Esters **2b** and **3b**

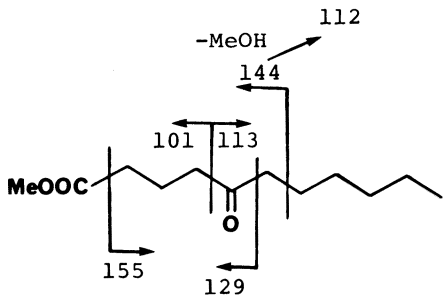

Fig. 3. Fragmentation in the EI-MS of **3b**

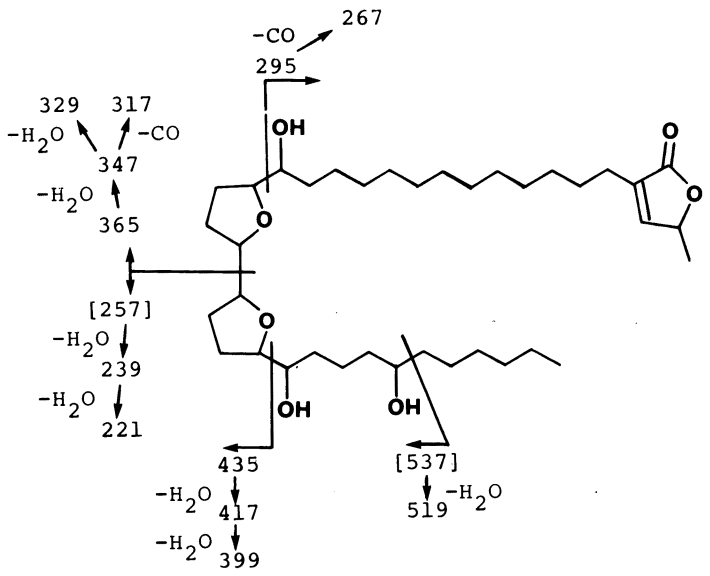

Fig. 4. Fragmentation in the EI-MS of **1**

The fragment ions in brackets were not detected. Ions at m/z 604 ($M-H_2O$), 586 ($M-2\times H_2O$), and 568 ($M-3\times H_2O$) were observed. FAB-MS showed the $[M+H]^+$ ion at m/z 623.

C-15, -24, and -28. It follows that the structure of squamocin is represented by **1**. The absolute and relative stereochemistry of **1** is unknown.

The EI-MS data of **1** are summarized in Fig. 4. Occurrence of the fragment ions arising from the cleavage of the C-15/C-16, C-19/C-20, and C-23/C-24 bonds, as in the case of related compounds,[3] strongly supported the presumptive bis-tetrahydrofuran ring. *In vitro* cytotoxic activity of squamocin was examined, and the ID_{50} value was 0.58 μg/ml against L1210 cells.

Experimental

^1H- and ^{13}C-NMR spectra were recorded on a JEOL FX-200 or Bruker 400 spectrometer in $CDCl_3$ with tetramethylsilane as an internal reference. IR spectra were determined on a JASCO IR-810 spectrometer. UV spectra were obtained on a Shimadzu UV 200 spectrometer. GC-MS and direct-inlet MS (70 eV) were obtained with a Shimadzu GC-MS DF 9020. FAB and FD-MS were obtained with a JEOL JMX-300 mass spectrometer.

Isolation of Squamocin (1)——Pulverized seeds of *A. squamosa* L., 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 petrol and chromatographed over silica gel. Elution of the column with solvents of increasing polarity furnished a nearly homogeneous thick oil from the $CHCl_3$–methanol (97 : 3) fractions. Rechromatography of this oil on silica gel yielded pure **1** (2.2 g), $[\alpha]_D^{20}$ +0.15 (c = 1.7, methanol). IR $\nu_{max}^{CHCl_3}$: 3680, 3585, 3460, 3015, 2940, 2855, 1755 cm^{-1}. UV λ_{max}^{MeOH}: 215 nm (log ε 3.5). *Anal.* Calcd for $C_{37}H_{66}O_7$: C, 71.20; H, 10.87. Found: C, 71.34; H, 10.68. This sample solidified in a refrigerator, mp below 30 °C.

Jones Oxidation of 1——A solution of **1** (100 mg) in dry acetone (20 ml) was titrated with Jones reagent, the end point being a persistent brown color. The organic solvent from the reaction mixture was evaporated with a stream of nitrogen and the residue was taken up in water and extracted with CH_2Cl_2. The extract was washed with water, dried and evaporated to dryness to furnish an amorphous powder (**2a** and **3a**, 12 mg). A part of the amorphous powder was analyzed by GC-MS after ethereal CH_2N_2 treatment. The GC-MS chromatogram (OV-1 capillary column) showed two peaks corresponding to **2b** and **3b**. EI-MS: **2b** *m/z* (relative intensity): 306 (M − H_2O, 3), 292 (M − MeOH, 21), 274 (M − H_2O − MeOH, 5), 251 (C_{11}–C_{12} fission, 17), 209 (10), 195 (24), 181 (10), 167 (13), 153 (16), 139 (18), 112 (C_3–C_4 fission-H, 97), 55 (100); **3b** *m/z*: 183 (M − OMe, 10), 157 (1), 155 (7), 144 (30), 129 (30), 112 (64), 101 (43), 43 (100). For the assignment of the fragment ions of **3b**, see Fig. 3. The ^{13}C-NMR spectrum of the product mixture showed high- and low-intensity signals (peak intensity, *ca.* 2 : 1 ratio). Carbon signals with low intensity were attributed to **3a**, and are identical with those of authentic **3a**. Further purification of the product mixture by silica gel chromatography (eluted with ethyl acetate–methanol 20 : 1) gave pure **2a** as an oil. IR $\nu_{max}^{CDCl_3}$: 3030, 2935, 2855, 1755, 1715 cm^{-1}. ^1H- and ^{13}C-NMR data for **2a** are listed in Table I. ^{13}C-NMR data for purified **2a** were in complete agreement with the high-intensity signals mentioned above. FD-MS of **2a** showed *m/z*: 311 $[M+H]^+$; EI-MS (direct inlet) *m/z*: 292 (M − H_2O, 15), 274 (M − 2 × H_2O, 4), 251 (6), 209 (8), 195 (18), 181 (17), 112 (C_3–C_4 fission-H, 100), 55 (90).

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