Coumarinolignans from the Seeds of *Annona squamosa* Linn.

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Abstract: Phytochemical investigations of *Annona squamosa* seeds have led to the isolation of three lignans consisting of coumarin moiety, cleomiscosin A(1), cleomiscosin B(2) and cleomiscosin C(3). Their structures were arrived at by detailed spectroscopic analysis. Cleomiscosin A and cleomiscosin B are position isomer.

Keywords: Phytochemical investigations, Seeds, Cleomiscosin, Spectroscopic analysis

Introduction

A. squamosa Linn. is commonly known as the custard apple. It is native of West Indies and has been naturalized throughout India in plains as well as on hills^{1,2}. *A. squamosa* L. is a well known edible tropical fruit and its seeds exhibit insecticidal and abortifacient properties³. *A. Squamosa* consists of a variety of compounds *e.g.* amino acids⁴ monoterpenes⁵, sesquiterpenes⁶, kaurene⁷, steroids⁸ *etc.* Our previous studies have resulted in the isolation of about 30 acetogenins from the seeds of *A. squamosa*, *eg.* Squamostanal-A⁹, Squamosin-O₁ and O₂¹⁰, squamosatin-A, Squamocins B-to-N¹¹ *etc.* In continuation of our work we report herein isolation and identification of compounds 1–3 from seeds of *Annona squamosa*.

Experimental

Extraction and purification

The air dried and pulverized seeds of *A. squamosa* were extracted in the Soxhlet extractor with MeOH (5L). The concentrated methanolic extract (150 g) was then fractionated into four different solvents *viz*; *n*-hexane, CHCl₃, Bu-OH and aqueous-MeOH (3:7) by mechanical stirring according to their polarity. The concentrated CHCl₃ soluble portion was then kept at 4 °C for 48 hour; a solid residue settled at the bottom of flask. It was then filtered and chromatographed over silica gel, 100 g. The column was eluted with CHCl₃:

MeOH (9: 1 to 5: 5) mixtures to afford three fractions 1, 2 and 3. Fraction 1 (5 g) was rechromatographed over silica gel column (50 g), using eluents benzene : EtOAc (8: 2–6: 4) to afford compound 1 & 2 (25 mg and 23 mg). Fraction 2 (3 g) was also subjected to column chromatography over silica gel (50 g) and eluted with the mixture of Benzene-EtOAc (6 : 4) to afford compound 3 (20 mg).

Table 1. ^1H - (500 MHz) and ^{13}C -NMR (125) data for 1 and 2 (in DMSO-d_6) and 3 (in CDCl_3).

	1		2		3	
	δ_c	δ_H	δ_c	δ_H	δ_c	δ_H
2	160.2		160.2		160.8	
3	113.4	6.33 (d, 9.5)	113.4	6.31 (d, 9.5)	114.4	6.35 (d, 9.5)
4	144.9	7.95 (d, 9.5)	145.0	7.95 (d, 9.5)	144.5	7.98 (d, 9.5)
5	101.0	6.90 (s)	101.1	6.94 (s)	101.0	6.93 (s)
6	145.4		145.5		146.3	
7	137.2		137.1		138.1	
8	131.8		132.1		132.5	
9	138.2		138.3		139.2	
10	111.4		111.3		111.8	
1'	126.8		126.9		126.5	
2'	112.2	7.02 (d, 1.8)	112.1	7.05 (d, 1.8)	106.1	6.76 (s)
3'	147.8		147.9		149.1	
4'	147.5		147.5		135.9	
5'	115.5	6.82 (d, 8.1)	115.6	6.83 (d, 8.1)	149.1	
6'	120.9	6.89 (dd, 8.1 & 1.8)	121.0	6.90 (dd, 8.1&1.8)	106.1	6.76 (s)
7'	78.0	4.98 (d, 7.9)	78.5	4.98 (d, 7.9)	77.7	4.99 (d, 7.9)
8'	76.4	4.31(ddd,7.9&3.5)	76.2	4.31(ddd,7.9, 3.7&2.3)	79.7	4.38(ddd,7.6, 4.5&3.1)
9'	60.0		60.1		60.8	
9'a	-	3.40(dd,11.3, 4.3&3.5)	-	3.64(ddd,11.5, 4.4&3.7)	-	3.68 (dd, 12.5 & 7)
9'b	-	3.67(ddd,11.3,4.3&2.2)	-	3.78 (ddd, 11.5, 4.4&2.3)	-	3.41 (dd, 12.5 & 3.5)
6-OCH ₃	55.9	3.78 (s)	55.9	3.84 (s)	56.1	3.77 (s)
3'-OCH ₃	56.0	3.77 (s)	56.1	3.79 (s)	56.4	3.77 (s)
5'-OCH ₃	-		-		56.4	3.93 (s)
-OCOCH ₃	-		-		20.7	
-OCOCH ₃	-		-		170.4	

Melting points were determined by using the Yazawa hot stage apparatus and are uncorrected. Optical rotations were recorded JASCO DIP-360 Polarimeter (cell length 5 cm). UV was recorded on JASCO UV / visible spectrophotometer (model no.7800). IR on JASCO FT-IR 5300 Spectrophotometer. NMR spectra were recorded on Bruker DRX 500 or JEOL GSX 500 with TMS as an internal reference. SiO_2 gel (Qualigens 60–120 mesh) was used for column chromatography and TLC was performed on pre-coated Silica gel G (Merck). All solvents were distilled prior to their use.

Compound 1

Colourless needles, m.p. 247–250 $^{\circ}\text{C}$, $\text{C}_{20}\text{H}_{18}\text{O}_8$ (M^+ at 386); $[\alpha]_{\text{D}}^{25} 0^{\circ}$ (c, 0.5 MeOH); UV (MeOH): λ_{max} 327 nm, 288 (sh); IR (KBr) ν_{max} 3443, 1705, 1613, 1580, 1516, 1444, 1423, 1381, 1346, 1280, 1236, 1191, 1161. (c.f. Table 1 for ^1H and ^{13}C -NMR data).

Compound 2

Colourless needles, m.p. 272-274 °C, C₂₀H₁₈O₈ (M⁺ at 386); [α]_D⁺ 0° (c, 0.5 MeOH); UV (MeOH): λ_{max} 327 nm, 288 (sh); IR (KBr) ν_{max} 3416, 1711, 1614. (c.f. Table 1 for ¹H and ¹³C-NMR data).

Compound 3

Colourless needles, m.p. 255 °C, C₂₁H₂₀O₉ (M⁺, 416) at 429.1173; [α]_D⁺ 0° (c 0.08); UV (MeOH): λ_{max} 232 (sh), 280 (sh), 328 nm; IR (KBr) ν_{max} 3420, 1720, 1685cm⁻¹. (c.f. Table 1 for ¹H and ¹³C-NMR data).

Results and Discussion

Compounds 1–3 have been identified by comparison of their spectroscopic and physical data with those reported in the literature^{12,13}. cleomiscosin A (1) was earlier isolated from a number of plants including *Cleome viscosa*¹⁴, *Simaba multiflora*¹⁵, *Soulamea soulameoides*^{16, 14}, *Matayaba arborescens*¹⁷, *Jatropha gossypifolia*¹⁸, *Hyoscyamus niger*¹² etc. cleomiscosin B (2) and cleomiscosin C (3) were earlier reported from *Cleome viscosa*^{19,20}, *Aesculus turbinata*²¹, *Hannoa kalineana*²², *Sasola laricifolia*²³ and in *Jatropha gossypifolia*²⁴. We report here the isolation of these compounds for the first time from the plant of annonaceae family.

Compound 1

¹H-NMR spectrum showed the presence of two doublets at δ_H 6.33 and 7.95 (1H, d, J= 9.5 Hz) for olefinic hydrogens and one aromatic proton signals at δ_H 6.90 (1H, s) is suggesting the presence of 6,7,8 trioxxygenated coumarin moiety in the compound 1. the spectrum also showed aromatic signals at δ_H 7.06, 6.89 and 6.82 for 1,3,4 tri-substituted aromatic nucleus, these three aromatic hydrogens taken along with the up-field hydrogen signals at δ_H 3.40 (1H, ddd, J= 3.5, 4.3 & 11.3 Hz), 3.67 (1H, ddd, J= 2.2, 4.3 & 11.3 Hz), 4.31 (1H, ddd, J= 3.5 & 7.9 Hz), 4.98 (1H, J= 9.8 Hz and 5.07 (1H, t, J= 4.3 Hz) suggested that these may be originated from a lignan part. Therefore compound 1 may be a coumarinolignan. The m.p. and other physical data of compound 1 were found comparable with cleomiscosin A. The identity of compound 1 and cleomiscosin A was established by direct comparison with authentic sample cleomiscosin A [mixed m.p, Co-TLC, identical HPLC].

Compound 2

The spectroscopic data of compound 2 were virtually identical with cleomiscosin A, but it differs from it in their physical properties like R_f on HPLC, the R_f value and the melting point. These allowed us to infer that compound 2 may be a regioisomer of cleomiscosin A i.e. Cleomiscosin B. Indeed it was found so when compared with an authentic sample of cleomiscosin B in respect to their R_f, R_f value and melting point.

Compound 3

It is a minor congener of cleomiscosins A and B. The ¹H-NMR spectrum of compound 3 showed signals for all the hydrogens as exhibited in compounds 1 and 2. The main difference was observed by the presence of one additional methoxy signal at δ_H 3.97 (3H, s) at the cost of one proton of ABX system present in the lignan part of the molecule. A comparison of the ¹H-NMR data of compound 3 with the relevant data of other reported coumarinolignans it was found to match well with cleomiscosin C. It showed identical R_f, R_f value and melting point when compared with an authentic sample of cleomiscosin C.

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