

## CHAPTER IV

### RESULTS AND DISCUSSION

The fresh stem bark of *Garcinia atroviridis* Griff. (12kg) was macerated with chloroform and then 95% ethanol. The Chloroform extract was separated by chromatographic technique to afford two pure compounds. The structure elucidation of the isolated compounds were based on data from UV, IR, MS, NMR and further confirmed by comparison with the data reported in literature, as discussed in this chapter.

#### 1. Structure Elucidation of GA-1

GA-1 was obtained as colorless needles from F-O4 (Table 6) by a column of Sephadex LH-20. It gave pink color with anisaldehyde-sulfuric acid spraying reagent which indicated that it was a steroidal compound as the previously mentioned (Robinstein *et al*, 1976).

The IR spectrum of GA-1 (Figure 2) suggested that functional groups of GA-1 were hydroxy group, methyl group, methylene group and alkyl group. (Table 9)

**Table 9** IR spectrum assignment of GA-1

Range of Absorption ( $\text{cm}^{-1}$ )	Intensity	Assignment
3500-3200	medium	O-H stretching of R-OH
2960-2860	high	C-H stretching of $\text{CH}_3$ , $\text{CH}_2$
1642	weak	C=C stretching of alkene
1465	medium	C-H bending of $\text{CH}_3$ (asymmetric), $\text{CH}_2$
1381	medium	C-H bending of $\text{CH}_3$ (symmetric)
1062	medium	C-O stretching
840, 802	weak	out-of-plane C-H bending of trisubstituted alkene

GA-1 could be assigned as a known steroid,  $\beta$ -sitosterol through analysis of its  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra. The  $^1\text{H}$ -NMR spectrum of GA-1 (Figures 3-5) showed the signals at  $\delta$  0.67-1.01 ppm which were the signals of methyl protons that substituted at C-18, C-19 and at side chain of the steroidal compounds. The signals at  $\delta$  1.10-2.30 ppm were the signals of methylene and methine protons of the steroid. The signal at  $\delta$  3.52 ppm (m) was the signal of the proton at C-3. The olefinic signal at  $\delta$  5.34 ppm (m) could be assigned as H-6 which was trisubstituted vinylic proton. The  $^{13}\text{C}$ -NMR spectrum (Figures 6-8) showed the signals of 3 quaternary, 9 methine, 11 methylene and 6 methyl carbons. The carbon assignments of GA-1 are shown in Table 10

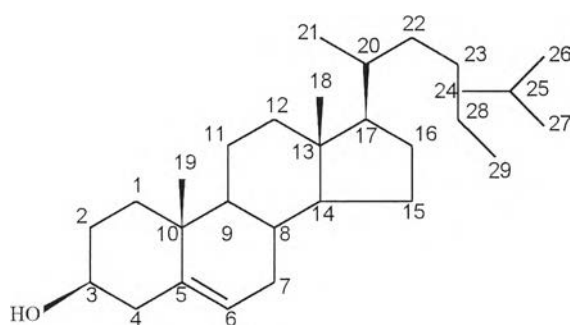
**Table 10** The <sup>13</sup>C-NMR chemical shifts of  $\beta$ -sitosterol and isolate GA-1

C position	Chemical shift (ppm)	
	$\beta$ -sitosterol	Isolate GA-1
1	37.31	37.27
2	31.57	31.67
3	71.69	71.80
4	42.25	42.31
5	140.76	140.77
6	121.59	121.71
7	31.92	31.91
8	31.92	31.91
9	50.17	50.14
10	36.51	36.51
11	21.11	21.10
12	39.81	39.78
13	42.33	42.31
14	56.79	56.78
15	24.32	24.31
16	28.26	28.25
17	56.11	56.07
18	11.87	11.87
19	19.40	19.40
20	36.17	36.15
21	18.82	18.78
22	33.95	33.95
23	26.13	26.06

**Table 10** (Continued)

C position	Chemical shift (ppm)	
	$\beta$ -sitosterol	Isolate GA-1
24	45.85	45.84
25	29.18	29.16
26	19.84	19.82
27	19.07	19.05
28	23.09	23.08
29	12.32	11.99

Both the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were in accordance with those published previously of  $\beta$ -sitosterol (Wright *et al.*, 1978). Therefore, it was concluded that GA-1 was  $\beta$ -sitosterol, the structure of which is shown below.

 $\beta$ -sitosterol

This structure was confirmed by the analyses of mass fragmentations. The EIMS spectrum (Figure 1) exhibited a molecular ion peak at  $m/z$  414 (32%) corresponding to molecular formula  $C_{29}H_{50}O$ . The proposed fragmentation patterns (Djerassi, 1969) are shown in Scheme 2.

## 2. Structure Elucidation of GA-2

GA-2 was obtained as yellow amorphous powder from fraction F-07 (Table 6) by repeated chromatographic technique.

In its HRMS, GA-2 displayed an ion as a molecular ion at  $m/z$  326.0781, corresponding to the formula  $C_{18}H_{14}O_6$  (calcd. for 326.0789 a.m.u.), and therefore suggesting a molecular formula of  $C_{18}H_{14}O_6$ .

The IR spectrum of GA-2 (Figure 10) exhibited absorption peaks of the hydroxyl group at  $3590-3100\text{ cm}^{-1}$ , the carbonyl group of  $\alpha,\beta$ -unsaturated ketone at  $1655\text{ cm}^{-1}$ , the olefinic at  $1579, 1482\text{ cm}^{-1}$ , the isopropyl group at  $1381, 1356, 1171\text{ cm}^{-1}$  and the ether linkage at  $1232\text{ cm}^{-1}$ .

The UV absorption spectrum of GA-2 (Figure 11) showing absorption maxima at 298 nm ( $\log\epsilon$  4.75) indicated a typical characteristic of the xanthone chromophore as previously mentioned. (Dahanayake *et al*, 1974 )

Compound GA-2 was identified as a new xanthone. The complete proton and carbon assignments were obtained by the analyses of  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts and their correlations observed in 2D-NMR spectra.

The  $^1\text{H-NMR}$  spectrum (Figure 12) showed signals for 2 methyl groups, 5 olefinic protons, and 3 hydroxyl protons. The lowest-field singlet signal at  $\delta$  12.70 ppm obtained in the  $^1\text{H-NMR}$  spectrum represented the chelated hydroxyl proton of C-5 due to intramolecular hydrogen-bonding between the hydroxyl group of C-5 and the carbonyl group of C-6. The singlet signals at  $\delta$  9.99 and 8.73 ppm were the hydroxy protons of C-8 and C-12, respectively.

Expansion of the  $^1\text{H-NMR}$  spectrum (Figure 13) exhibited signals indicative ABC type system. The proton at position 9 ( $\delta$  7.29 ppm, dd,  $J=9.16$ , 2.93 Hz) was *ortho*-coupled to H-10 ( $\delta$  7.50 ppm, d,  $J=9.16$  Hz) and *meta*-coupled (or long-range coupling *via* W-coupling) to H-7 ( $\delta$  7.40 ppm, d,  $J=2.93$  Hz).

The characteristic signals of two vinylic protons at  $\delta$  5.76 ppm (d,  $J=10.25$  Hz, H-3) and  $\delta$  6.62 ppm (d,  $J=10.25$  Hz, H-4) and six-proton signal at  $\delta$  1.44 ppm ( $\text{C-2}(\text{Me})_2$ ) indicated the presence of a 2, 2-dimethylchromene group (Sultanbawa and Surendrakumar, 1985).

The signals observed in its  $^{13}\text{C-NMR}$  spectrum (Figure 14) and DEPT spectra (Figure 15) revealed 1 carbonyl carbon, 5  $\text{sp}^2$  carbons, 10 quaternary carbons and 2 methyl groups.

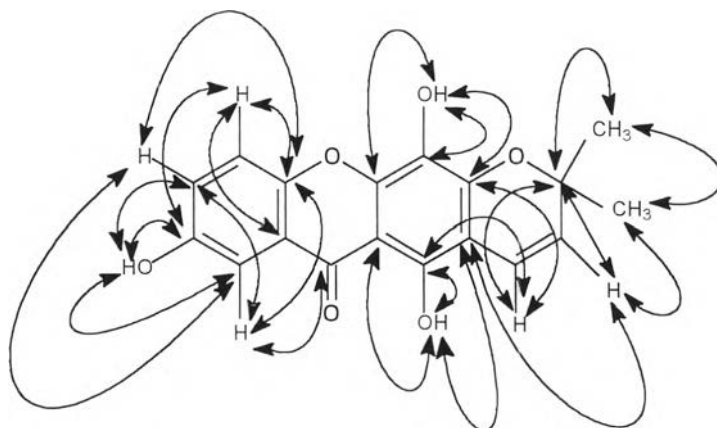
It could be inferred from above data that there are a number of alternative structures of GA-2 to be deduced : (a) 5,8,12-trihydroxy-2,2-dimethyl-2H,6H-pyrano[3,2-b]xanthene-6-one and (b) 5,9,12-trihydroxy-2,2-dimethyl-2H,6H-pyrano[3,2-b]xanthene-6-one. In order to evaluate the structure more

explicitly, detailed examinations of the HMBC and HMQC of GA-2 were undertaken.

With the aid of HMQC spectrum (Figure 18), the upfield methyl group ( $\delta$  27.76 ppm) could be assigned to Me<sub>2</sub> at C-2. The other protonated carbons were C-3 at 128.54, C-4 at 114.91, C-7 at 107.90, C-9 at 124.68 and C-10 at 119.11 ppm.

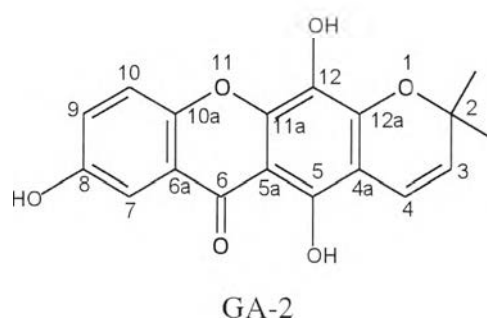
The assignments of quaternary carbons of GA-2 were made from the HMBC spectrum (Figure 16) as well as their chemical shifts. The signal at 180.38 ppm was assigned to the carbonyl carbon at C-6. The oxygen connected sp<sup>3</sup> carbon at 77.94 ppm was assigned to C-2 which also showed long-range correlation with H-3 ( $\delta$  5.76 ppm). The other quaternary carbons assigned were summarized in Table 11.

<sup>13</sup>C-<sup>1</sup>H correlations observed in the HMBC and HMQC spectra of GA-2 are shown below.



The explicit assignments of carbon and proton signals with long-range correlations HMBC were summarized in Table 11

The structure of GA-2 was finally confirmed by the analyses of mass fragmentation (Scheme 3) from its EIMS spectrum (Figure 9). The molecular ion peak ( $M^+$ ) at  $m/z$  326 corresponded to the molecular formula of  $C_{18}H_{14}O_6$ . The base peak at  $m/z$  311 ( $M^+ - 15$ ) resulted from  $\alpha$ -cleavage leading to loss of a methyl group. The structure of GA-2 is shown below.

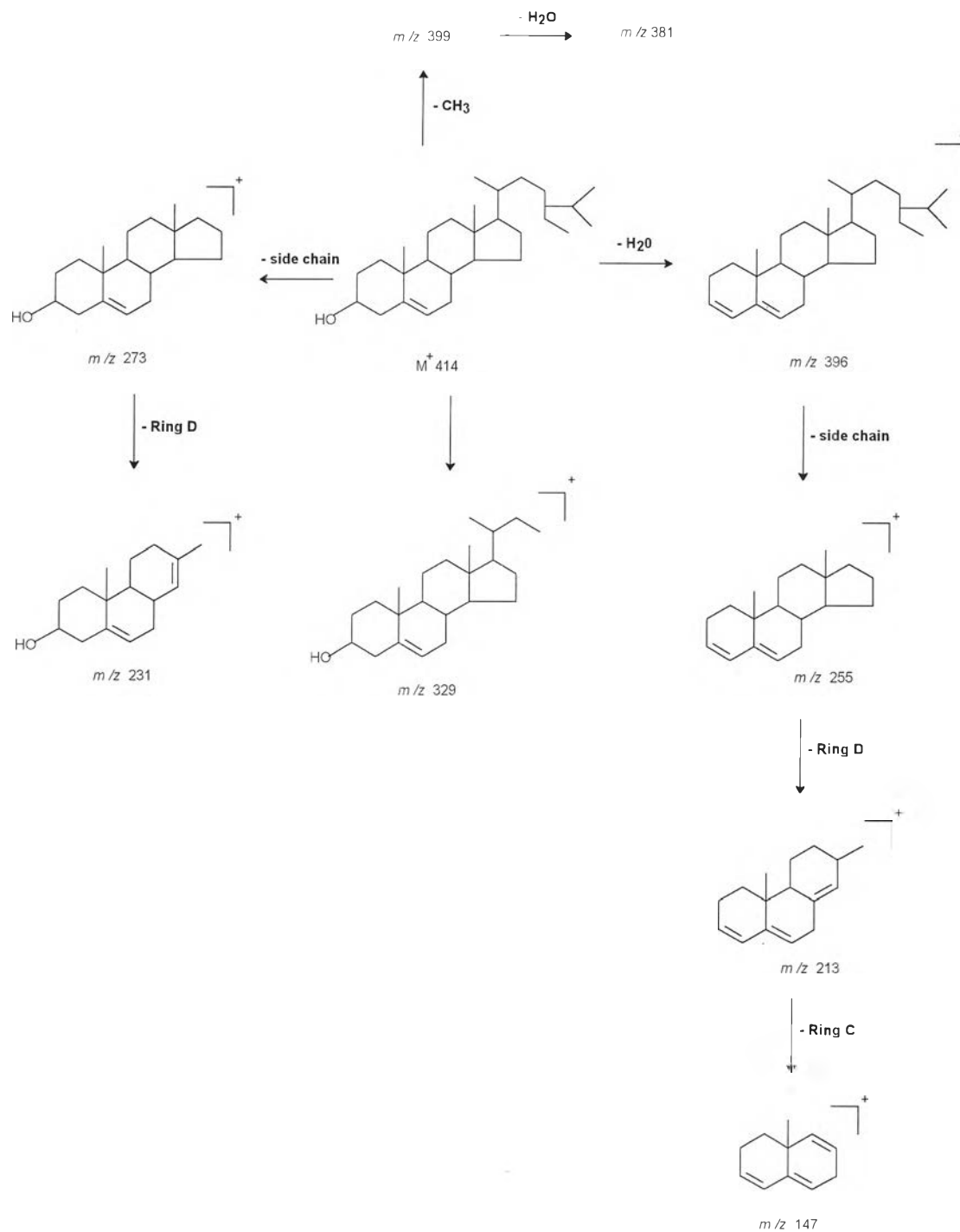


These results strongly support the proposed structure of GA-2 as 5,8,12-trihydroxy-2,2-dimethyl-2H,6H-pyrano[3,2-b]xanthene-6-one, a new xanthene, trivial named atroviridin.

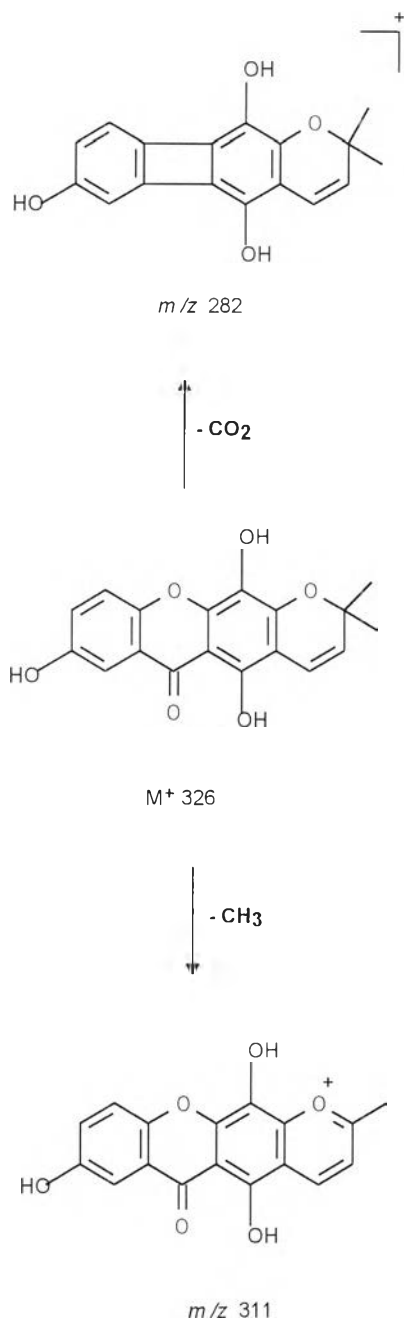


**Table 11** The carbon and proton assignments with long-range correlations observed in HMBC spectrum.

Position	$\delta$ C (ppm)	$\delta$ H (ppm)	HMBC
2	77.94	-	2-(Me) <sub>2</sub> , H-3, H-4
3	128.54	5.76 (d, J=10.25)	2-(Me) <sub>2</sub>
4	114.91	6.62 (d, J=10.25)	-
4a	103.48	-	H-3, H-4, 5-OH
5	148.74	-	5-OH
5a	102.28	-	5-OH
6	180.38	-	H-7
6a	120.13	-	H-10
7	107.90	7.40 (d, J=2.93)	8-OH, H-9
8	153.91	-	-
9	124.68	7.29 (dd, J=2.93, 9.16)	H-7, 8-OH
10	119.11	7.50 (d, J=9.16)	-
10a	149.08	-	H-7, H-9, H-10
11a	145.10	-	12-OH
12	125.35	-	-
12a	148.47	-	12-OH
2-(Me) <sub>2</sub>	27.76	1.44	2-(Me) <sub>2</sub> , H-3
5-OH	-	12.70(s)	-
8-OH	-	9.99(s)	-
12-OH	-	8.73(s)	-



**Scheme 2** Mass fragmentation of GA-1



**Scheme 3** Mass fragmentation of GA-2