CHAPTER 2

EXPERIMENTAL

2.1 Plant Materials

The rhizomes part of *Globba reflexa* (753.4 g) were collected from Doi Sutep-Pui National Park, Chiang Mai province in May 2008. The sample was identified by J. F. Maxwell of the CMU Herbarium, where a voucher specimen was deposited (CMU10-Nuchnipa Nuntawong). The rhizomes were air died.

2.2 General Experimental Procedures

2.2.1 Column Chromatography (CC)

Adsorbent: Silica gel 60 for layer chromatography with particle size 0.063-0.200 mm (Merck, 7734) was used in the experiments.

Packing procedures: Slurry packing.

Sample loading: The sample was dissolved in a small amount of suitable organic solvent, mixed with a small amount of silica gel 60 with particle size 0.063-0.200 mm (Merck, 7734), air dried and added gently onto the top of column.

Elution: The column was eluted with suitable solvent by gradient system after loading of the sample.

2.2.2 Flash Column Chromatography (FCC)

Adsorbent: Silica gel with particle size less than 0.063 mm (Merck, 7729) was used in the experiments.

Packing procedures: Slurry packing.

Sample loading: The sample was dissolved in a small amount of suitable organic solvent, mixed with a small quantity of silica gel 60 with particle size less than 0.063 mm (Merck, 7729), air dried and added gently onto the top of column.

Elution: The column was eluted with suitable solvent by gradient system after loading of the sample.

2.2.3 Thin Layer Chromatography (TLC)

Adsorbent: Silica gel 60 F_{254} aluminum plate (Merck) size 1 × 5 cm and 2.5 × 5 cm.

Detection of Chromatographic plate

- Ultraviolet light: The compound which contains unsaturated bonds especially conjugated system was show spot under UV light at 254 nm.

- Developing reagents: Anisaldehyde reagent contituted of *p*-anisaldehyde (1.13 mL), concentrated sulfuric acid (1.7 mL), acetic acid (0.5 mL) and ethanol (51.8 mL). The organic compounds were showed specific colors of spot with this reagent after heating at 90-110 °C for 2-4 minutes.

2.2.4 Physical Property

Melting points were determined on an Electrothermal melting point apparatus and were uncorrected. The temperature was given in degree Celsius. Optical rotations were obtained using a AP-300 automatic polarimeter.

2.2.5 Spectroscopy

2.2.5.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

Compounds were dissolved in $CDCl_3$ and subjected to ^1H and ^{13}C NMR analysis using a Bruker AVANCE 400 NMR spectrometer, operated at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts were recorded in ppm (δ) using TMS as internal standard.

2.2.5.2 Infrared (IR) Spectroscopy

Compounds were dissolved in CH_2Cl_2 and recorded as the thin film technique. After that, they were analyzed using a FT-IR spectrometer (Tensor 27).

2.2.5.3 Gas Chromatography/ Mass Spectroscopy (GC-MS)

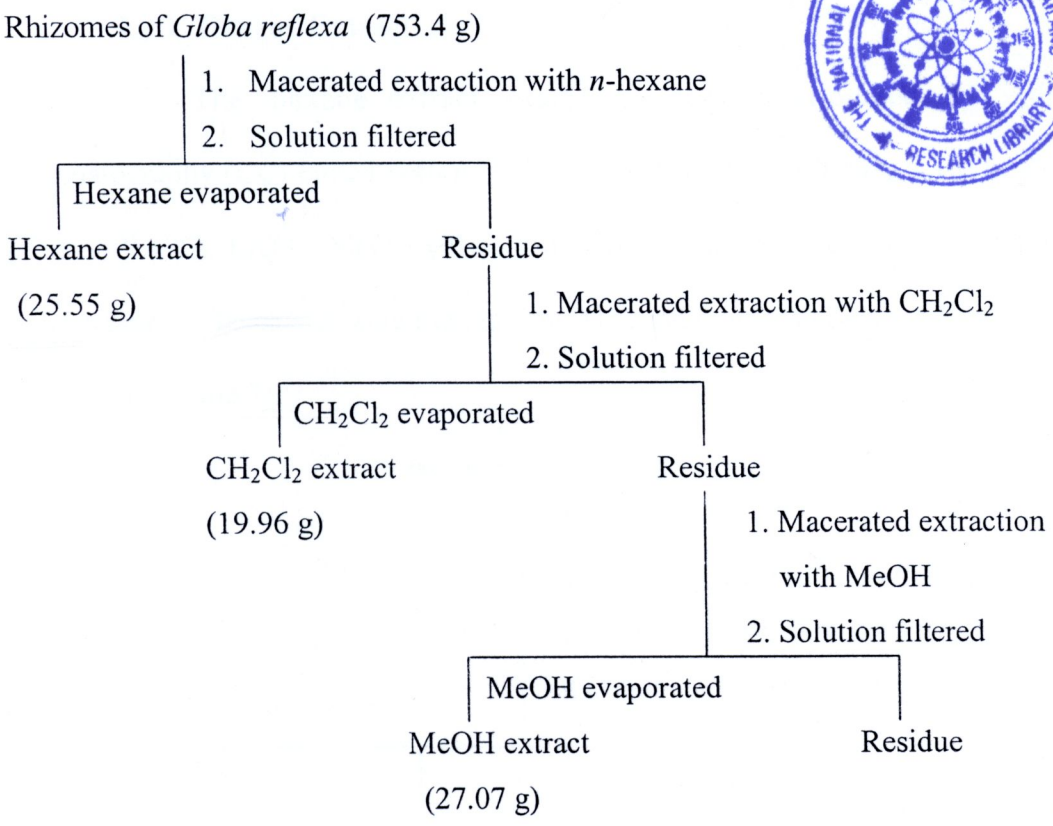
Compounds were dissolved in CH_2Cl_2 and analyzed using a Agilent-HP 5973 Mass Spectrometer interfaced with Agilent-HP 6890 AT-1 GC capillary column (30 m x 0.25 mm, 0.25 μm film thickness).

The oven temperature was programmed from 35-270 °C at the rate of 2 °C/min with final hold 12.5 min, using helium gas as a carrier gas. Individual components were identified by Wiley 275 and NIST database matching. Relative percentage of individual components were calculated based on GC peak

areas without using correction factors. The injector and detector temperatures were 200 °C and 230 °C, respectively. MS were taken at 70 eV with mass range of *m/z* 35-550.

2.3 Extraction and Isolation

The air dried rhizomes of *Globba reflexa* (753.4 g) were coarsely ground and then soaked in *n*-hexane for 5 days at room temperature for three times. The combined filtrate was evaporated to dryness under reduced pressure at temperature 40-45 °C. The crude *n*-hexane extract was obtained as a brownish viscous oil (25.55 g). Then the residue was successively extracted with CH_2Cl_2 and MeOH by the same proceduce as crude *n*-hexane extract to afford the crude CH_2Cl_2 extract as a brownish viscous oil (19.96 g) and crude MeOH extract as a dark-brown gum (27.07 g). The extraction procedure is shown in Scheme 1.



Scheme 1 Extraction of the rhizomes of *Globba reflexa.*

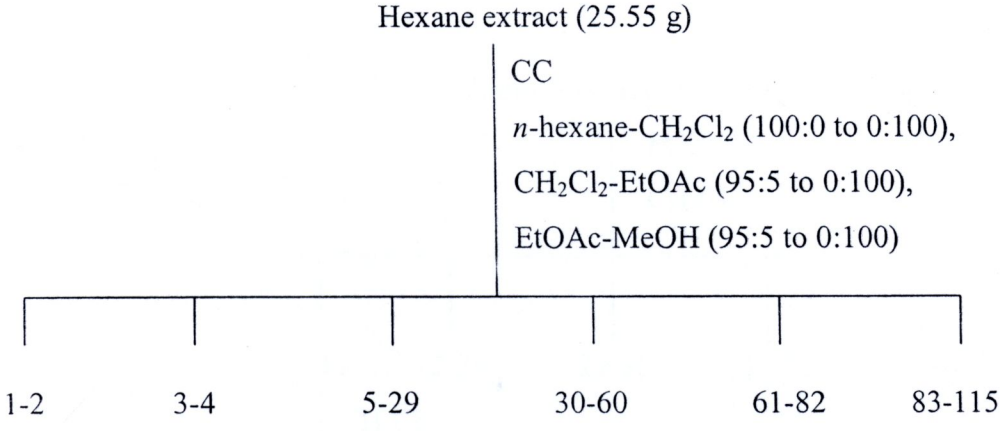
The crude extracts of the rhizomes of *Globba reflexa* with various solvents were show in table 1.

Table 1 The crude extracts of the rhizomes of *Globba reflexa* with various solvents.

Solvent extract	Appearance	Weight (g)	% w/w of the dried rhizome
Hexane	brownish viscous oil	25.55	3.39
CH_2Cl_2	brownish viscous oil	19.96	2.65
MeOH	dark-brown gummy	27.07	3.59

2.3.1 Hexane Extract

The hexane extract (25.55 g) was fractionated by column chromatography (CC) eluted with *n*-hexane-CH_2Cl_2 (100:0 to 0:100), CH_2Cl_2-EtOAc (95:5 to 0:100), EtOAc-MeOH (95:5 to 0:100) with increasing amount of the more polar solvent. The eluates were examined by TLC and 6 groups of eluting fractions were obtained (Scheme 2).

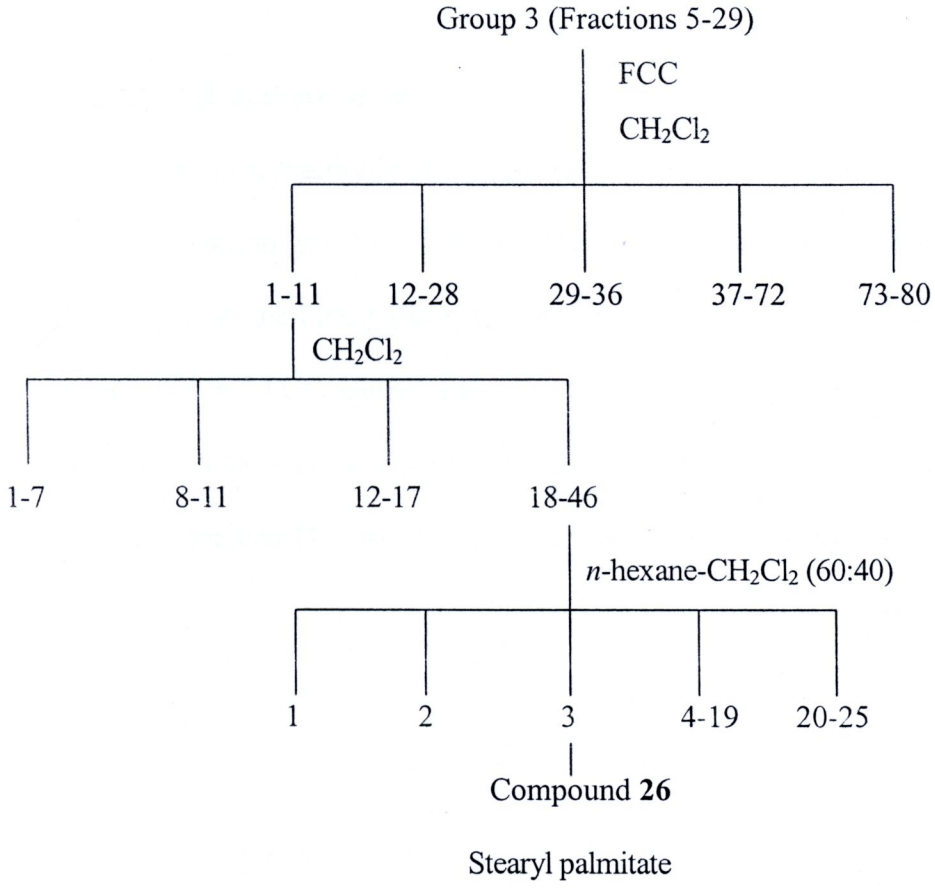


Gr. 1, fractions 1-2 (0.2791 g)
Gr. 2, fractions 3-4 (0.0431 g)
Gr. 3, fractions 5-29 (2.1644 g)
Gr. 4, fractions 30-60 (2.8049 g)
Gr. 5, fractions 61-82 (4.6043 g)
Gr. 6, fractions 83-115 (10.2041 g)

Scheme 2 Fractionation of the hexane extract of *Globba reflexa*.

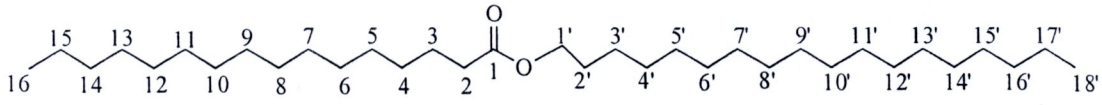
Group 3 (fractions 5-29)

This combined fraction was rechromatographed over silica gel eluted with CH_2Cl_2 to give 5 fractions. Subfraction 1 (fractions 1-11) was separated by FCC eluted with CH_2Cl_2 to give 4 fractions. Subfraction 4 (fractions 18-46) was purified by FCC eluted with *n*-hexane-CH_2Cl_2 (60:40) to give compound **26** as white amorphous solid (11.7 mg) (Scheme 3).



Scheme 3 Fractionation of Group 3 (fractions 5-29) of the hexane extract.

Stearyl palmitate (**26**)



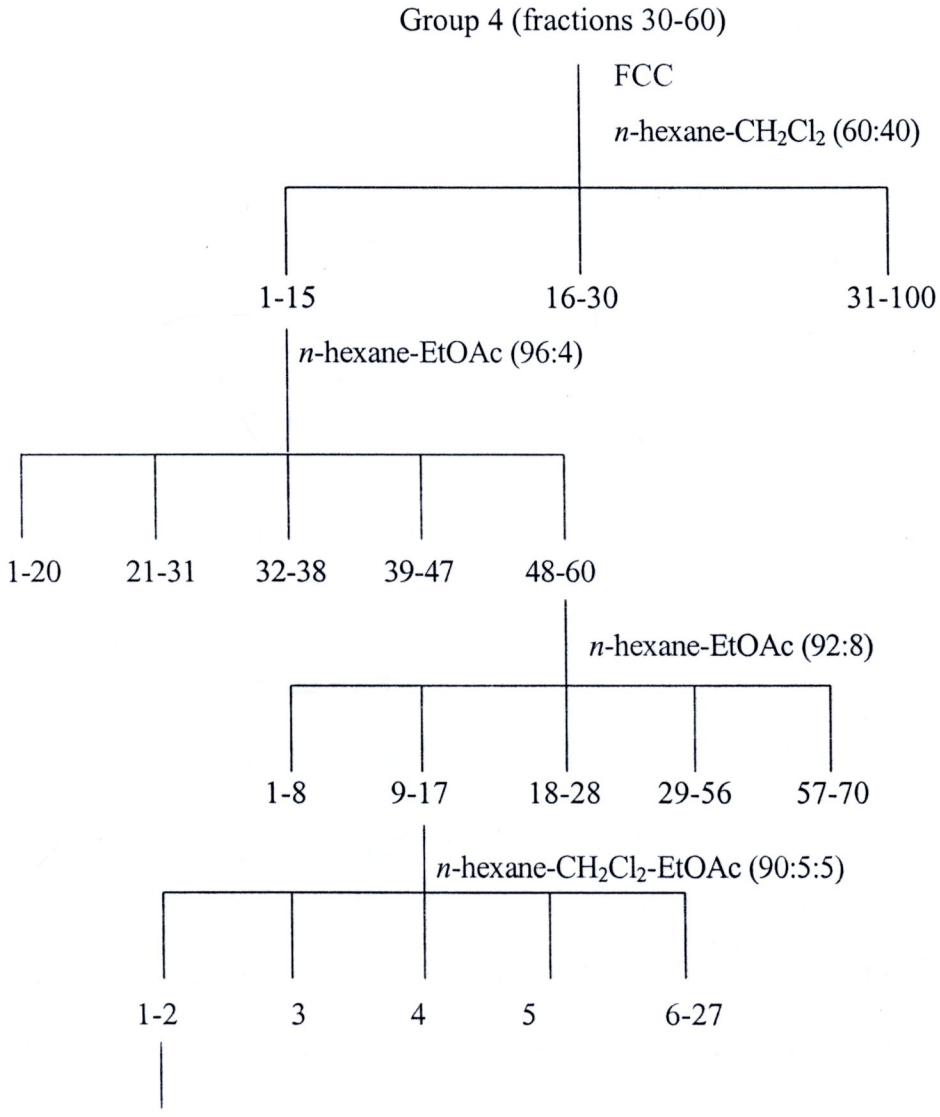
IR: ν_{max} 740, 1264, 1377, 1464, 1729, 2852 and 2924cm^{-1}

^{1}H NMR and ^{13}C NMR data are given in Table 2.

EIMS: *m/z* 508 $[M]^{+}$(10); 57 (78), 97 (43),239 (14), 257 (100), 283 (4), 297 (7).

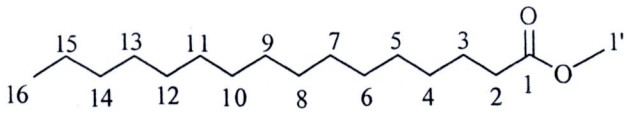
Group 4 (fractions 30-60)

This combined fraction was rechromatographed over silica gel eluted with *n*-hexane-CH_2Cl_2 (60:40) to give 3 fractions. Subfraction 1 (fractions 1-15) was separated by FCC eluted with *n*-hexane-EtOAc (96:4) to give 5 fractions. Subfraction 5 (fractions 48-84) was separated by FCC eluted with *n*-hexane-EtOAc (92:8) to give 5 fractions. Subfraction 2 (fractions 9-17) was purified by FCC eluted with *n*-hexane-CH_2Cl_2-EtOAc (90:5:5) to give compound **27** as white amorphous solid (12.9 mg) (Scheme 4).



Scheme 4 Fractionation of Group 4 (fractions 30-60) of the hexane extract.

Methyl palmitate (**27**)



M.p. 32.0-35.0 °C.

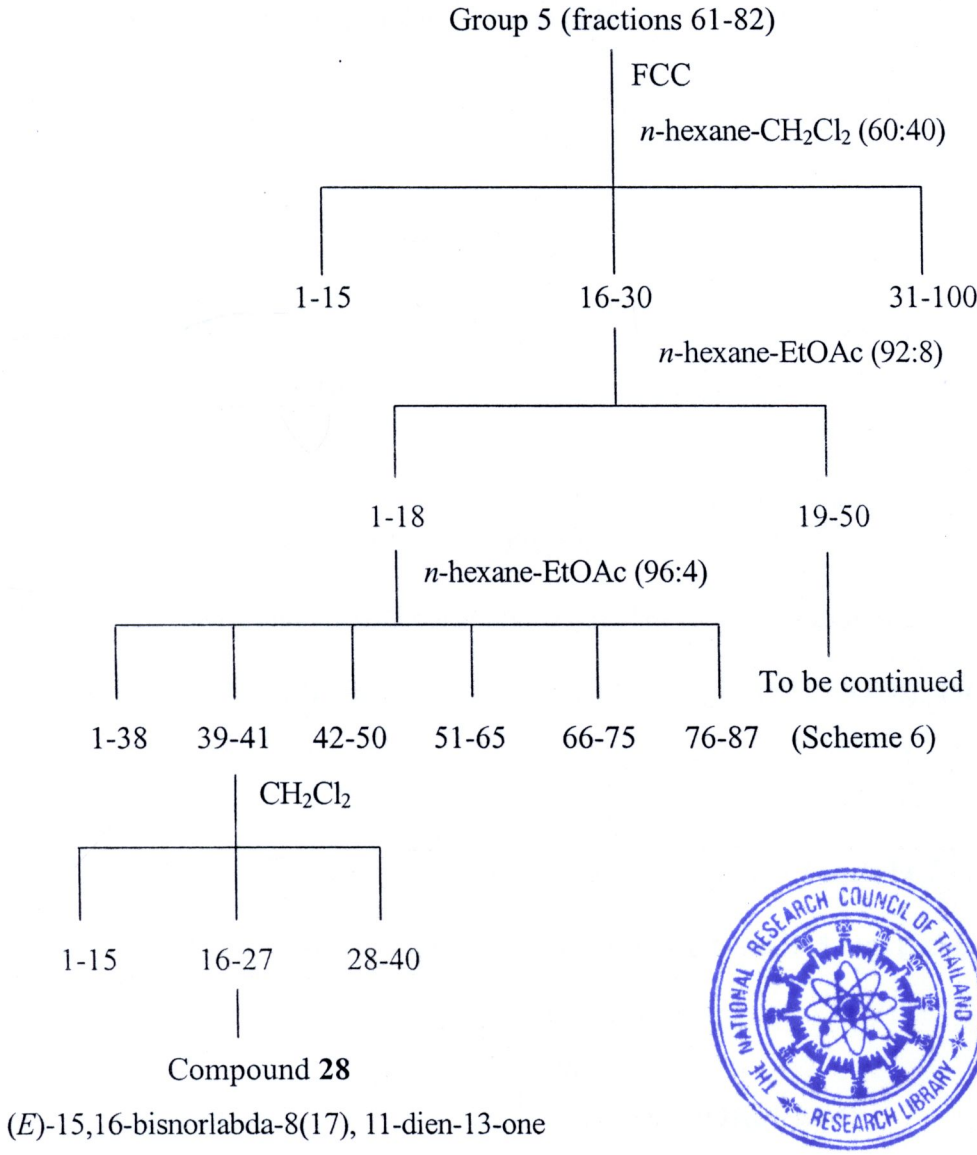
IR: ν_{max} 750, 1170, 1385, 1463, 1742, 2854 and 2924 cm^{-1}

^{1}H NMR and ^{13}C NMR data are given in Table 3.

EIMS: *m/z* 270 $[M]^{+}$ (8); 43 (28), 55 (24), 74 (100), 87 (69), 143 (18), 227 (12)

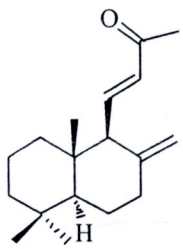
Group 5 (fractions 61-82)

This combined fraction was rechromatographed over silica gel eluted with *n*-hexane-CH_2Cl_2 (60:40) to give 3 fractions. Subfraction 2 (fractions 16-30) was separated by FCC eluted with *n*-hexane-EtOAc (92:8) to give 2 fractions. Subfraction 1 (fractions 1-18) was separated by FCC eluted with *n*-hexane-EtOAc (96:4) to give 6 fractions. Subfraction 2 (fractions 39-41) was purified by FCC eluted with CH_2Cl_2 to give compound **28** as colorless amorphous solid (8.1 mg) (Scheme 5).



Scheme 5 Fractionation of Group 5 (fractions 61-82) of the hexane extract.

(*E*)-15,16-bisnorlabda-8(17),11-dien-13-one (**23**)



$[\alpha]_D^{27}$ -29.24 (*c* 0.2735, $CHCl_3$)

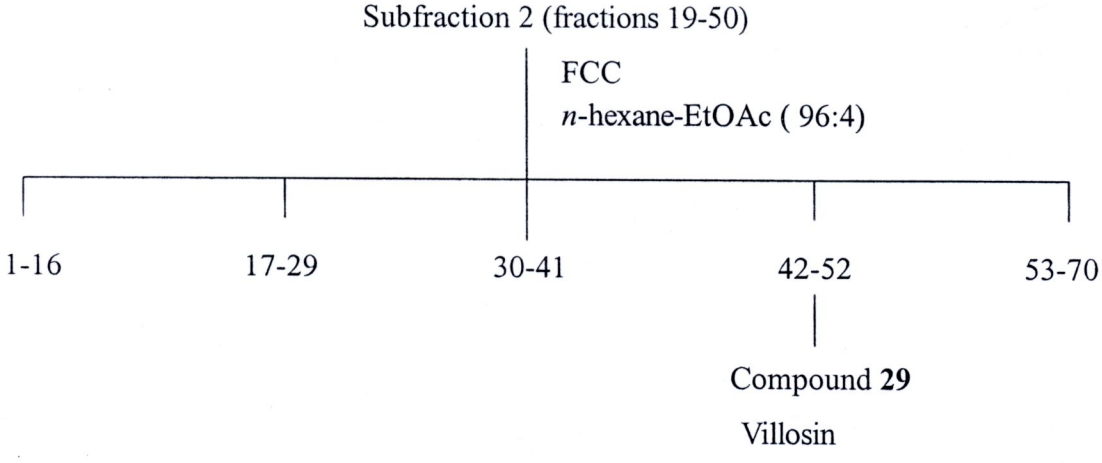
M.p. 146.0-147.0 °C.

IR: ν_{max} 2936, 1664, 1456, 1361, 1258, 998, 898 and 601 cm^{-1}

^1H NMR, ^{13}C NMR and HMBC correlations data are given in Table 4.

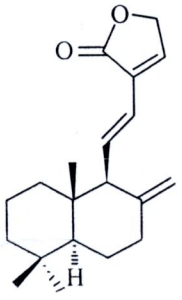
EIMS: *m/z* 260 $[M]^+$ (47) ; 245 (16), 217 (35), 137 (60), 81 (92), 69 (30), 43(58)

Subfraction 2 (fractions 19-50) was purified by silica gel FCC (*n*-hexane-EtOAc, 96:4) to give compound **29** as white amorphous solid (9.5 mg) (Scheme 6).



Scheme 6 Fractionation of subfraction 2 (fractions 19-50) of Scheme 5.

Villosin (**29**)



$[\alpha]_D^{27}$ -93.00 (*c* 0.043, $CHCl_3$).

M.p. 124.5-125.0 °C.

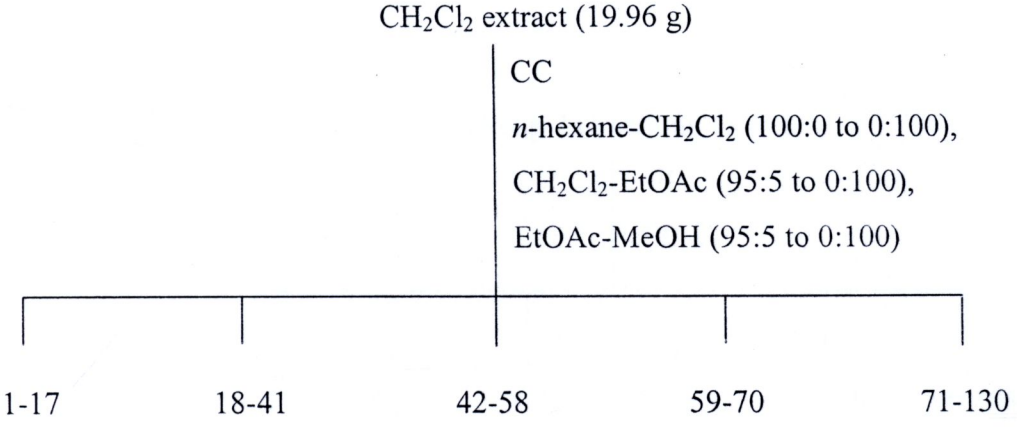
IR: ν_{max} 2925, 1754, 1640, 1443, 1382, 1086, 1052, 947, 902 and 833 cm^{-1}.

^1H NMR, ^{13}C NMR and HMBC correlations data are given in Table 5.

EIMS: *m/z* 300 $[M]^+$ (19) ; 285 (10), 257 (4), 189 (6), 137 (100), 123 (22), 55 (15), 41 (20)

2.3.2 CH_2Cl_2 Extract

The CH_2Cl_2 extract (19.96 g) was fractionated by column chromatography (CC) eluted with *n*-hexane-CH_2Cl_2 (100:0 to 0:100), CH_2Cl_2-EtOAc (95:5 to 0:100), EtOAc-MeOH (95:5 to 0:100) with increasing amount of the more polar solvent. The eluates were examined by TLC and 5 groups of eluting fractions were obtained (Scheme 7).

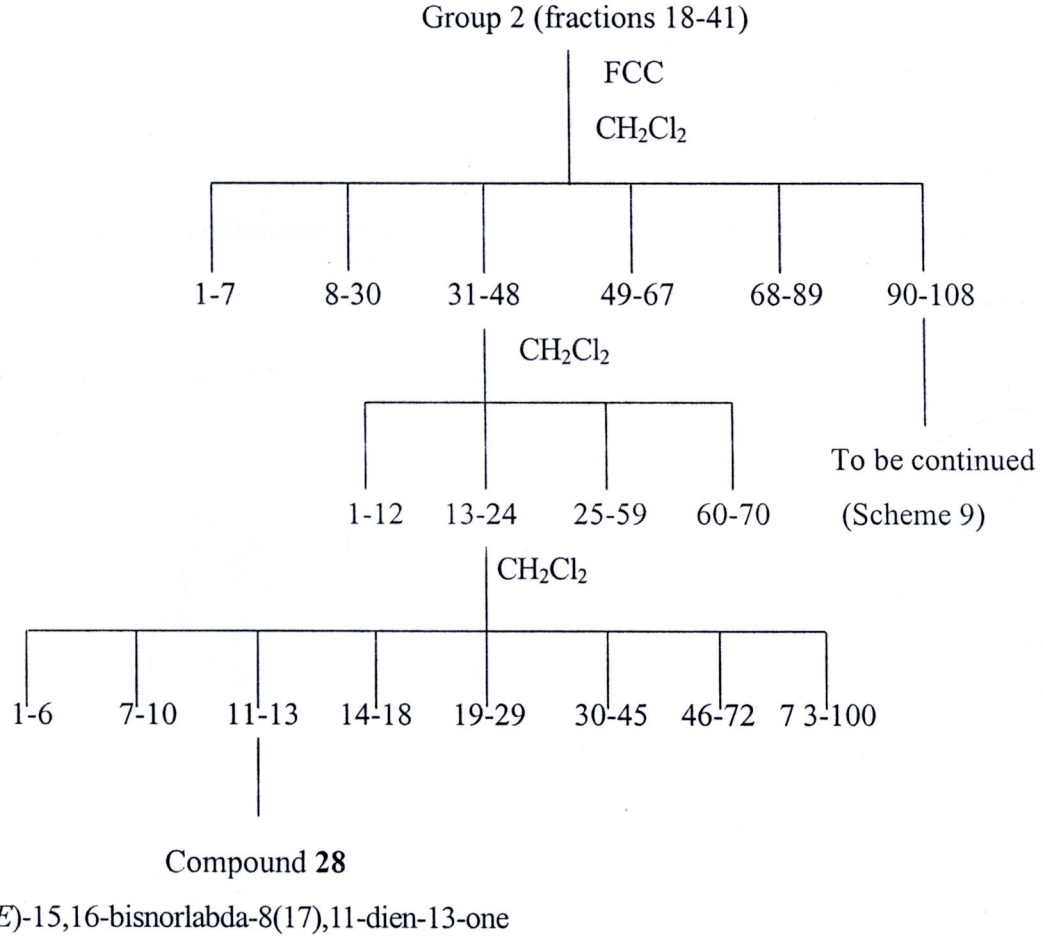


Gr. 1, fractions 1-17 (0.2791 g)
Gr. 2, fractions 18-41 (0.0431 g)
Gr. 3, fractions 42-58 (2.1644 g)
Gr. 4, fractions 59-70 (2.8049 g)
Gr. 5, fractions 71-130 (4.6043 g)

Scheme 7 Fractionation of the CH_2Cl_2 extract of *Globba reflexa*.

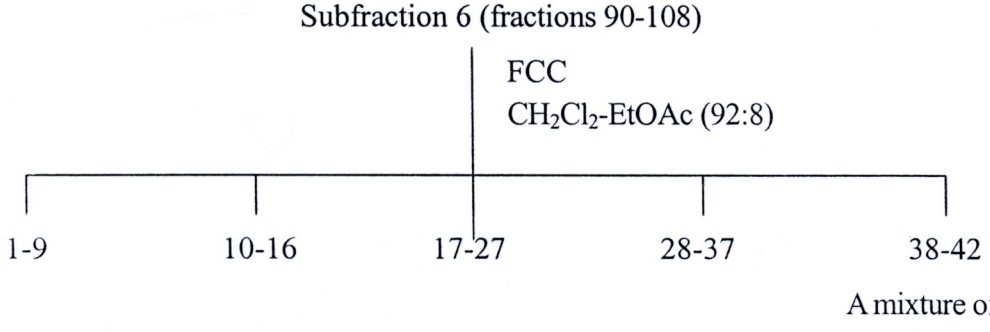
Group 2 (fractions 18-41)

This combined fraction was rechromatographed over silica gel eluted with CH_2Cl_2 to give 6 fractions. Subfraction 2 (fractions 18-41) was separated by FCC eluted with CH_2Cl_2 to give 6 fractions. Subfraction 3 (fractions 31-48) was separated by FCC eluted with CH_2Cl_2 to give 4 fractions. Subfraction 2 (fractions 13-24) was purified by FCC eluted with CH_2Cl_2 to give compound **28** as colorless amorphous solid (26.3 mg) (Scheme 8).



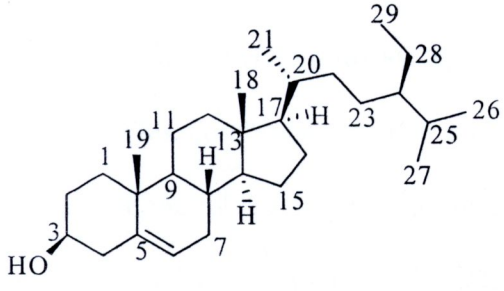
Scheme 8 Fractionation of Group 2 (fractions 18-41) of the CH_2Cl_2 extract.

Subfraction 6 (fractions 90-108) was purified by FCC eluted with CH_2Cl_2-EtOAc (92:8) to give a mixture of compounds **13**, **30** and **31** as colorless crystals (59.8 mg) (Scheme 9).

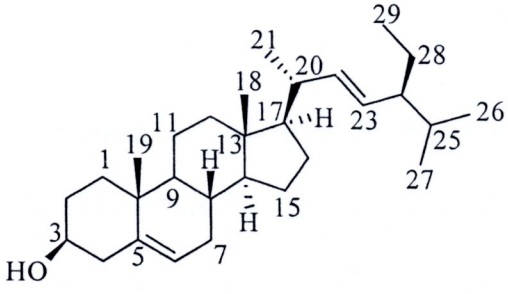


Scheme 9 Fractionation of subfraction 6 (fractions 90-108) of Scheme 8.

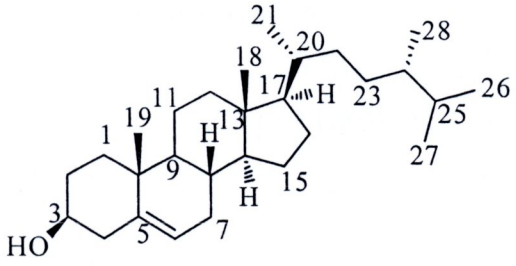
β–sitosterol (**13**), stigmasterol (**30**) and campesterol (**31**)



β–sitosterol (**13**)



stigmasterol (**30**)



campesterol (**31**)

M.p. 144-146 °C.

IR: ν_{max} 3422, 2947, 2866, 1511, 1459, 1119, 1376, 1248, 1031, 832, 732, 524 cm^{-1}.

^1H NMR data are given in Table 6.

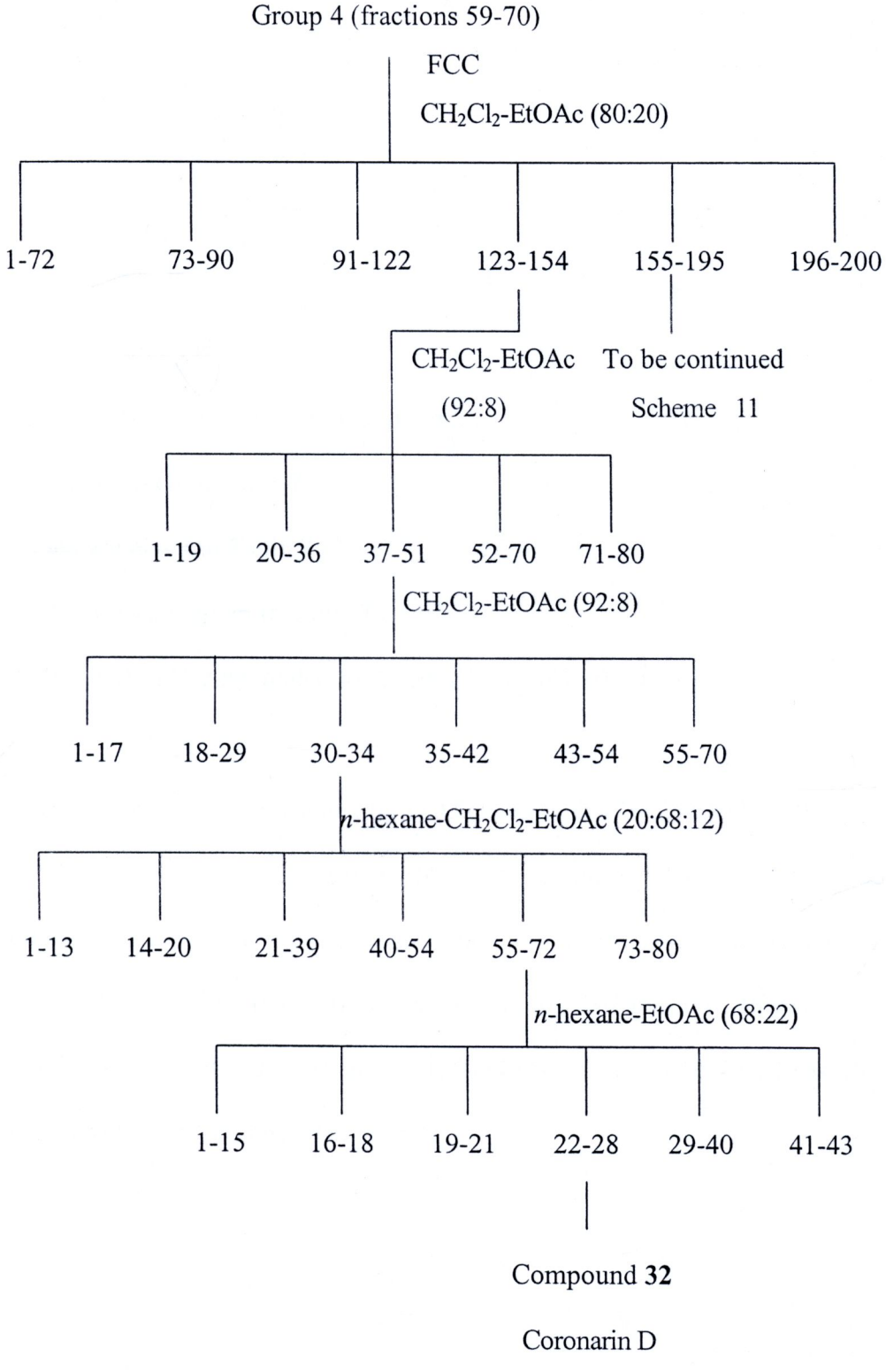
EIMS: *β*–sitosterol (**13**) *m/z* 414 $[M]^+$ (98) ; 43 (78), 105 (52), 145 (61), 213 (44), 255 (48), 273 (26), 303 (46), 329(50), 396 (100)

stigmasterol (**30**) *m/z* 412 $[M]^+$ (75) ; 55 (98), 83 (71), 105 (50), 159 (52), 185 (11), 207 (100), 255(73), 300 (32), 351 (30), 394 (47)

campesterol (**31**) *m/z* 400 $[M]^+$ (82) ; 42 (72), 105 (53), 207 (100), 255 (40), 289 (49), 315 (50), 382 (77)

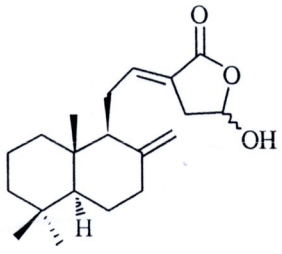
Group 4 (fractions 59-70)

This combined fraction was rechromatographed over silica gel eluted with CH_2Cl_2-EtOAc (80:20) to give 6 fractions. Subfraction 4 (fractions 123-154) was separated by FCC eluted with CH_2Cl_2-EtOAc (92:8) to give 5 fractions. Subfraction 2 (fractions 20-36) was separated by FCC eluted with CH_2Cl_2-EtOAc (92:8) to give 6 fractions. Subfraction 3 (fractions 30-94) was separated by FCC eluted with *n*-Hexane-CH_2Cl_2-EtOAc (20:68:12) to give 6 fractions. Subfraction 5 (fractions 55-72) was purified by FCC eluted with *n*-Hexane-EtOAc (68:22) to give compound **32** as pale yellow oil (3.3 mg) (Scheme 10).



Scheme 10 Fractionation of Group 4 (fractions 59-70) of the CH_2Cl_2 extract.

Coronarin D (**32**)



$[\alpha]_D^{27}$ +35.25 (*c* 0.033, $CHCl_3$).

IR: ν_{max} 3381, 2938, 1737, 1458, 1177, 943 cm^{-1}

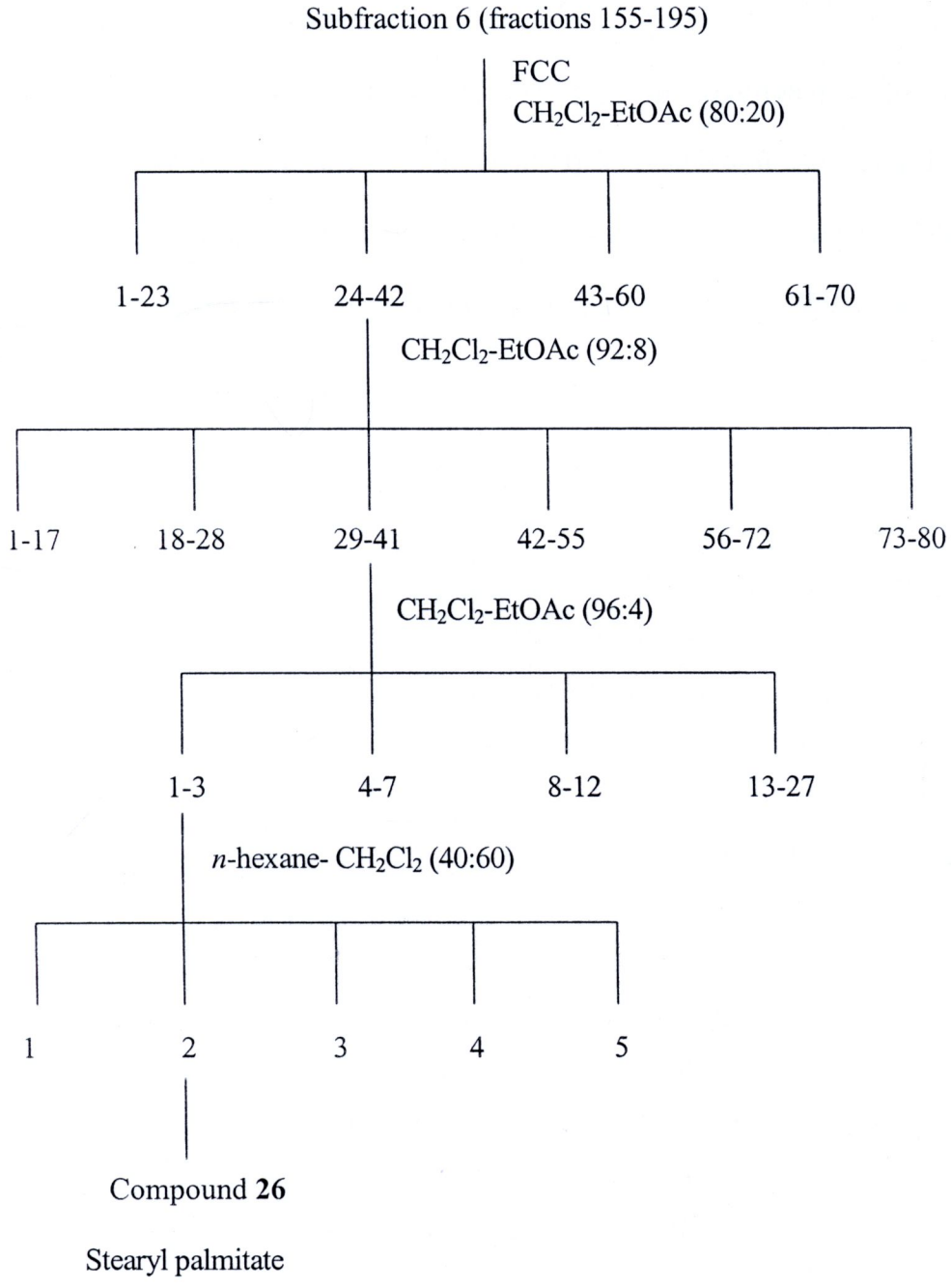
^1H NMR data are given in Table 7.

^{13}C NMR data are given in Table 7.

HMBC correlations are given in Table 7.

HREIMS: $[M+Na]^+$ 341.2090 (calcd for $C_{20}H_{30}O_3Na$, 341.2093)

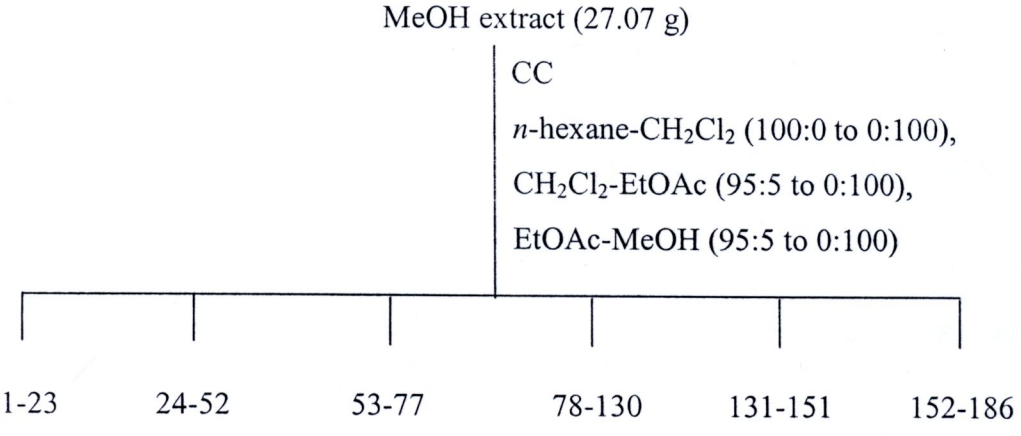
Subfraction 5 was separated by FCC eluted with CH_2Cl_2-EtOAc (80:20) to give 4 fractions. Subfraction 2 (fractions 24-42) was separated by FCC eluted with CH_2Cl_2-EtOAc (92:8) to give 6 fractions. Subfraction 3 (fractions 29-41) was separated by FCC eluted with CH_2Cl_2-EtOAc (96:4) to give 4 fractions. Subfraction 1 (fractions 1-3) was purified by FCC eluted with *n*-Hexane- CH_2Cl_2 (40:60) to give compound **26** as white amorphous soild (11.7 mg) (Scheme 11).



Scheme 11 Fractionation of subfraction 5 (fractions 155-195) of Scheme 10.

2.3.3 MeOH Extract

The MeOH extract (27.07 g) was fractionated by column chromatography (CC) eluted with *n*-hexane-CH_2Cl_2 (100:0 to 0:100), CH_2Cl_2-EtOAc (95:5 to 0:100), EtOAc-MeOH (95:5 to 0:100) with increasing amount of the more polar solvent. The eluates were examined by TLC and 6 groups of eluting fractions were obtained (Scheme 12).

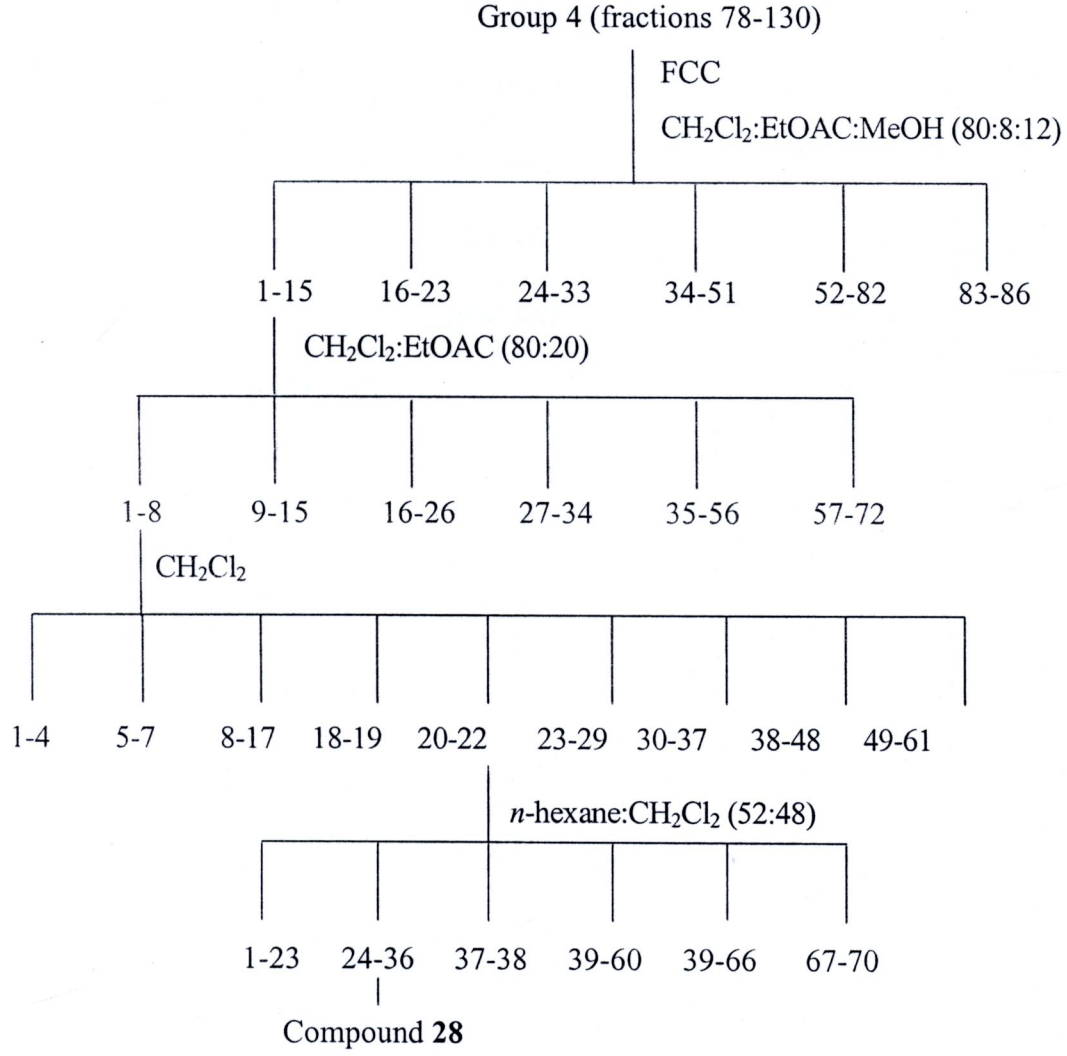


Gr. 1, fractions 1-23 (0.1169 g)
Gr. 2, fractions 24-52 (0.2471 g)
Gr. 3, fractions 53-77 (0.4498 g)
Gr. 4, fractions 78-130 (10.1446 g)
Gr. 5, fractions 131-151 (3.1458 g)
Gr. 6, fractions 152-186 (9.3684 g)

Scheme 12 Fractionation of the MeOH extract of *Globba reflexa*.

Group 4 (fractions 78-130)

This combined fraction was rechromatographed over silica gel eluted with CH_2Cl_2:EtOAC:MeOH (80:8:12) to give 6 fractions. Subfraction 1 (fractions 1-15) was separated by FCC eluted with CH_2Cl_2:EtOAC (80:20) to give 6 fractions. Subfraction 1 (fractions 1-8) was separated by FCC eluted with CH_2Cl_2 to give 9 fractions. Subfraction 4 (fractions 20-22) was purified by FCC eluted with *n*-Hexane:CH_2Cl_2 (52:48) to give compound **28** as colorless amorphous solid (3.5 mg) (Scheme 13).



(*E*)-15,16-bisnorlabda-8(17),11-dien-13-one

Scheme 13 Fractionation of Group 4 (fractions 78-130) of the MeOH extract.

Table 2 ^{1}H and ^{13}C NMR data of compound **26**

Positions	δ ^{1}H (Mult., *J* in Hz)	δ ^{13}C
1		173.36
2	2.28 (*m*)	34.43
3	1.62(*m*)	25.05
4-15	1.25 (*m*)	22.71-31.94
16-Me	0.87 (*m*)	14.12
1′	4.05 (*t, 6.7*)	64.42
2′	1.60(*m*)	29.12
3′-17′	1.26 (*m*)	22.71-31.94
18′-Me	0.88 (*m*)	14.13

Recorded in $CDCl_3$

Table 3 ^{1}H and ^{13}C NMR data of compound **27**

Positions	δ ^{1}H (Mult., J in Hz)	δ ^{13}C
1		120.16
2	2.32 (*m*)	34.88
3	1.65 (*m*)	25.18
4-14	1.29 (*m*)	29.18-29.70
15	1.31 (*m*)	22.69
16-Me	0.88 (*m*)	14.11
1′-Me	3.65 (*s*)	51.9

Recorded in $CDCl_3$

Table 4 ^{1}H and ^{13}C NMR data of compound **28**

Positions	δ ^{1}H (Mult., *J* in Hz)		δ ^{13}C		HMBC
	Comp.**28**	Ref.[28]	Comp.**28**	Ref.[28]	
1	α 1.04	1.04	40.86	36.6	C-2, 3, 5, 10, 20
	(obscured signal)	(*ddd*,12.5,12.5,5.0)			
	β 1.36 (*m*)	1.40 (*m*)			
2	α 1.40 (*m*)	1.55 (*m*)	18.99	19.0	C-1, 3, 4
	β 1.53 (*m*)	1.55 (*m*)			
3	α 1.19 (*m*)	1.21	42.09	42.0	C-1, 2, 4, 5
		(*ddd*,12.5,12.5,5.0)			
	β 1.45 (*m*)	1.39 (*m*)			
4			33.54	33.6	
5	1.10 (*dd*, 12.5, 2.6)	1.11 (*dd*,12.5,2.5)	54.45	54.4	C-4, 6, 9, 10
6	α 1.39 (*m*)	1.46 (*m*)	23.23	23.2	C-5, 7, 8, 10
	β 1.71 (*m*)	1.74 (*m*)			
7	α 2.10	2.11	36.61	40.9	C-5 , 6, 8, 9, 17
	(*dt*, 12.5, 4.8)	(*ddd*,12.5,12.5,5.0)			
	β 2.46	2.45(*m*)			
	(overlapping)				
8			148.61	148.5	C-8, 12, 17, 20
9	2.46 (*br d*, 10.0)	2.48 (*d*, 10.0)	60.79	60.8	
10			39.33	39.3	
11	6.87	6.88 (*dd*, 16.0,10.0)	146.72	146.5	C-9, 10, 12, 13
	(*dd*, 15.8, 10.0)				
12	6.06 (*d*, 15.8)	6.08 (*d*, 16.0)	133.57	133.5	C-9, 11, 13, 1'
13			198.18	197.9	
17a	4.40 (*br d*, 1.3)	4.42 (*d*, 1.5)			
17b	4.78 (*br d*, 1.3)	4.81 (*d*, 1.5)	108.61	108.6	C-7, 8, 9
18	0.89 (*s*)	0.91 (*s*)	33.57	33.6	C-3, 4, 5, 19
19	0.82 (*s*)	0.86 (*s*)	21.92	21.9	C-3, 4, 5, 18
20	0.89 (*s*)	0.91 (*s*)	15.11	15.1	C-1, 5, 9, 10
1'	2.28 (*s*)		27.21	27.2	

Recorded in $CDCl_3$

Table 5 ^{1}H and ^{13}C NMR data of compound **29**

Positions	$\delta^{1}H$ (Mult., *J* in Hz)		$\delta^{13}C$		HMBC
	Comp.**29**	Ref.[31]	Comp.**29**	Ref.[31]	
1	α 1.03 (*m*)	0.97 (*ddd*, 13.5, 13.5)	40.89	40.8	C-2, 10
	β 1.39 (*m*)	1.50 (*br d*, 13.5)			
2	α 1.42 (*m*)	1.34 (*m*)	19.19	19.0	C-1, 3
	β 1.51 (*m*)	1.52 (*m*)			
3	α 1.17 (*m*)	1.18 (*br t*, 12.9)	42.29	42.2	C-2,4, 18, 19
	β 1.38 (*m*)	1.36			
4			33.66	33.5	
5	1.10 (*dd*, 12.5, 2.5)	1.07 (*dd*,12.5,2.3)	54.75	54.7	C-4, 6, 7, 10
6	α 1.41 (*m*)	1.36(*ddt*,13.1,4.8)	23.45	23.3	C-5, 7, 8, 10
	β 1.70 (*m*)	1.68			
7	α 2.09 (*dt*, 13.4, 5.1)	2.05	36.83	36.7	C-5, 6, 8, 9, 17
		(*ddd*,13.2,13.1,4.8)			
	β 2.45	2.41			
	(*ddd*, 13.4,4.0, 2.0)	(*ddd*,13.2,2.3,1.8)			
8			149.50	149.3	
9	2.38 (*br d*, 10.1)	2.34 (*br d*, 10.0)	62.28	62.1	C-8, 10, 20
10			39.31	39.2	
11	6.90 (*dd*, 15.8, 10.1)	6.87 (*dd*, 15.7, 10.1)	136.92	136.8	C- 8, 9, 10, 12
12	6.14 (*d*, 15.8)	6.08 (*d*, 15.7)	120.75	120.6	C-11, 13, 14, 16
13			129.56	129.5	
14	7.16 (*br s*)	7.13 (*br s*)	142.55	142.3	C-12, 13, 15, 16
15	4.81 (*br s*)	4.78 (*br s*)	69.69	69.5	C-13, 14
16			172.46	172.2	
17a	4.50 (*s*)	4.48 (*br s*)	108.49	108.3	C-7, 8, 9
17b	4.76 (*s*)	4.73 (*br s*)			
18	0.90 (*s*)	0.86 (*s*)	33.66	33.5	C-3, 4, 5, 19
19	0.88 (*s*)	0.81 (*s*)	22.03	21.9	C-3, 4, 5, 18
20	0.84 (*s*)	0.85 (*s*)	15.14	15.0	C-1, 5, 9, 10

Recorded in $CDCl_3$

Table 6 ^{1}H and ^{13}C NMR data of a mixture of compounds **13**, **30** and **31**

Position	*β*-Sitosterol			
	^{1}H	^{1}H Ref.[34]	^{13}C	^{13}C Ref.[34]
3	3.53 (*m*)	3.52 (*m*)	71.8	71.8
6	5.36 (*m*)	5.358 (*br s*)	140.8	140.8
18	0.68 (*s*)	0.68 (*s*)	11.6	11.9
19	1.02 (*s*)	1.01 (*s*)	19.2	19.4
21	0.93 (*s*)	0.92 (*d*, 6.4)	18.5	18.8
26	0.86 (*d*)	0.814 (*d*, 6.5)	19.2	19.8
27	0.83 (*d*)	0.833 (*d*, 6.5)	19.3	19.3
29	0.85 (*d*)	0.845 (*t*, 7.5)	12.3	12.2

Recorded in $CDCl_3$

Table 7 ^{1}H and ^{13}C NMR data of compound **32**

Positions	$\delta^{1}H$ (Mult., *J* in Hz)		$\delta^{13}C$		HMBC
	Comp.**32**	Ref.[32]	Comp.**32**	Ref.[32]	
1	α 1.05 (*m*)	1.05 (overlapping)	39.26	39.23	C-2, 10
	β 1.68 (*m*)	1.79 (overlapping)			
2	α 1.52 (*m*)	1.50 (overlapping)	19.33	19.27	C-1, 3
	β 1.58 (*m*)	1.58 (*ddd, 13, 3, 3*)			
3	α 1.20 (*m*)	1.20 (overlapping)	42.00	41.96	C-2,4, 18, 19
	β 1.40 (*m*)	1.41 (*br d*, 13)			
4			35.57	33.58	
5	1.10 (*td*, 12.6, 3.0)	1.12(*br d*,13)	55.33	55.33	C-4, 6, 7, 10
6	α 1.32 (*dd*, 12.8, 4.1)	1.33 (*dd*,13,4)	24.10	24.05	C-5, 7, 8, 10
	β 1.74 (*br t*, 2.3)	1.74 (overlapping)			
7	α 2.00 (*dt*, 12.9, 4.6)	2.00 (overlapping)	37.79	37.75	C-5, 6, 8, 9, 17
	β 2.37 (*m*)	2.39 (overlapping)			
8			147.93	147.88	
9	1.85 (*br d*, 10.1)	1.87 (obscured signal)	56.16	56.12	C-8, 10, 20
10			39.46	39.42	
11	2.2 (*m*)/2.33 (*m*)	2.20/2.35 (overlapping)	24.54	25.48	C- 8, 9, 10, 12
12	6.75 (*m*)	6.75 (*m*)	143.74	143.50	C-11, 13, 14, 16
13			124.17	124.13	
14	2.70 (*m*)/3.01 (*m*)	2.71 (*br d*, 17)/3.04(*m*)	33.57	33.51	C-12, 13, 15, 16
15	5.91 (*dd*, 3.3, 2.3)	5.93 (*m*)	95.96	95.94	C-13, 14
16			170.50	169.97	
17a	4.38 (*s*)/4.40 (*s*)	4.35 (*s*)/4.81 (*s*)	107.35	107.28	C-7, 8, 9
17b	4.82 (*s*)/4.80 (*s*)	4.40 (*s*)/4.83 (*s*)			
18	0.89 (*s*)	0.88 (*s*)	33.57	33.58	C-3, 4, 5, 19
19	0.80 (*s*)	0.82 (*s*)	22.73	21.67	C-3, 4, 5, 18
20	0.72 (*s*)	0.72 (*s*)	14.36	14.30	C-1, 5, 9, 10

Recorded in $CDCl_3$

2.4 Biological Activities

2.4.1 Cytotoxicity Assays

The cytotoxicity assays against human oral epidermoid carcinoma (KB), human breast adenocarcinoma cancer (MCF-7) and human small cell lung cancer (NCI-H187) were performed employing colorimetric method.[20] The standard drugs doxorubicin showed IC_{50} values against these cell lines at 0.082 , 0.663 and 0.053 μg/mL respectively, and ellipticine exhibited IC_{50} values against KB, NCI-H187 and noncancerous Vero cells at 0.302, 0.440 and 1.345 μg/mL. The cytotoxicity evaluation of compounds **28**, **29** and **32** is shown in Table 8.

2.4.2 Antiplasmodial Assay

The antiplasmodial activity was assessed against *Plasmodium falciparum* K1 using the parasite lactate dehydrogenase (pLDH) assay.[21] The inhibition profiles and IC_{50} determinations of the pLDH assay were directly comparable to those determined by the radioactive uptake and microscopic methods. The standard drugs dihydroartemisinine and mefloquine showed IC_{50} values of 1.37 nM and 0.0351 μM respectively. The anti-malaria assay of compounds **28**, **29** and **32** is shown in Table 9.

2.5.3 Antimycobacterial Assay

The antimycobacterial activity was assessed against *Mycobacterial tuberculosis* H37Ra using the green fluorescent protein microplate assay (GFPMA).[22] The lowest drug concentration effecting and inhibition of ≥ 90 % was considered the MIC. The standard drugs, rifampicine, streptomycin, isoniazid, oflaxacin and ethambutol showed MIC values of 0.003-0.025, 0.156-0.313, 0.023-0.046, 0.391-0.781 and 0.234-0.469 μg/mL respectively. The antimycobacterial assay of compounds **28**, **29** and **32** is shown in Table 9.

Table 8 Cytotoxic activities of compounds **28**, **29** and **32**

Compound	Cytotoxicity (IC_{50}, μg/ml)		
	MCF7[a]	KB[b]	NCl-H187[c]
28	49.22	36.99	21.67
29	18.01	6.85	2.12
32	16.25	40.01	25.72

[a] human breast adenocarcinoma cancer

[b] human oral epidermoid carcinoma

[c] human small cell lung cancer

Table 9 Antiplasmodial and antimycobacterial activities of compounds **28**, **29** and **32**

Compound	Antiplasmodial (IC_{50}, μg/ml)	Antimycobacterial (MIC, μg/ml)
28	Inactive	Inactive
29	Inactive	Inactive
32	No test	Inactive