Note: 1) The evaluation on the biological activity of some isolated compounds such as Cpd. 5, 7, 8, 12, 13 and 20 are in progress.

2) Some isolated compounds are obtained in unsufficient amount for evaluating their biological activity, such as Cpd.4, 11, 21 and 22.

3) The insect antifeedant activity results of Fraction III and Fraction X were presented in Scheme 2.4 and 2.5, respectively.

CHAPTER III

Results and Discussion

Various parts of Rhizophora apiculata Bl.* -barks, branches, flowers, hypocotyls, leaves, overground and underground supporting roots and heartwoods- were collected from the Klong Wat Mai district, Samutsongkarm province, Thailand during October 1985. Each specimen was extracted for phytochemical screening tests and for biological activity utilizing appropriate procedures** against fungi, bacteria and the boll weevil insect. The extraction of each plant part with dichloromethane and further with 95% ethanol found that the dichloromethane extract crude from the leaves gave the highest percentage yield, while the highest percentage yield of 95% ethanolic extract was obtained from the barks (see also Table 2.1).

3.1 Phytochemical Screening Tests

Phytochemical screening tests (62) on each part of R. apiculata revealed that all parts of this plant contained leucoanthocyanin; deoxy sugar (Keller-Kilani's reagent); steroids or

---

* Identified as R. apiculata Bl. by Associate Professor Amorn Ubonchonlaket and Associate Professor Pipat Patanaponbaiboon, Department of Botany, Chulalongkorn University.

** The bioassay experiments were carried out at Mississippi State University, USA. (see Topic 2.8)

triterpenoids (Liebermann-Burchard's reagent) as well as hydrolyzable and condensed tannins. Moreover, the ethanolic extract of the leaves, barks and branches gave a positive test for flavone compounds (see also Table 2.2).

3.2 Preliminary Bioassay Results

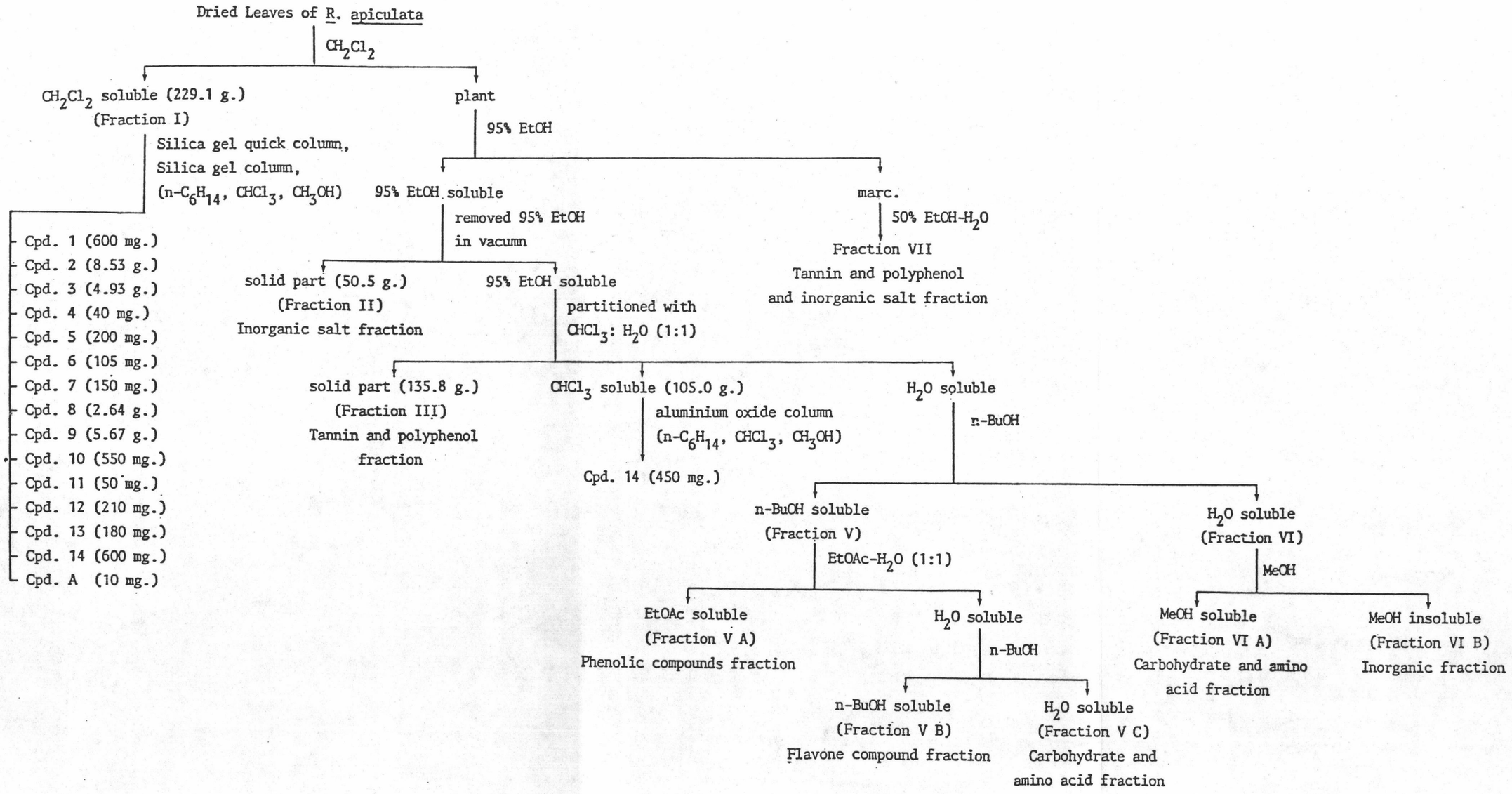
Preliminary bioassay experiments revealed that the ethanolic extract of the leaves gave an extremely large zone of inhibition against the fungus Helminthosporium teres. The dichloromethane and ethanolic extracts of the barks inhibited the bacteria Xanthramonas campestrous. The ethanolic extract of the branches showed antifungal activity against Pythium ultimum and antibacterial activity against Xanthramonas campestrous. The dichloromethane extract of the heartwoods revealed strong inhibition against the fungi Pythium ultimum and Rhizoctonia solani, and antibacterial activity against Xanthramonas campestrous (see also Table 2.3-2.4 and Scheme 2.4-2.5).

From the results of the preliminary bioassay study, the leaves and the heartwoods of this plant disclosed attractive bioassay results. Thus, these two parts were selected for investigation their chemical constituents and biologically active substances.

Part I Chemical Constituents of the Leaves of R. apiculata Bl.

The extraction of the leaves of R. apiculata with dichloromethane and 95% ethanol yielded dichloromethane soluble fraction (Fraction I, 4.60% wt. by wt. of plant sample) and 95% ethanol soluble fraction (10.28% wt. by wt.). During evaporating 95% ethanol in vacuum, white deposited solid was separated and labelled as Fraction II (0.78% wt. by wt.). The crude product of 95% ethanol soluble fraction was equilibrated with chloroform and water in a ratio 1:1 to gain an insoluble fraction (Fraction III, 2.09% wt. by wt.), a chloroform extract (Fraction IV, 1.22% wt. by wt.) and a water soluble part. The water soluble fraction was extracted further by n-butanol to afford a crude n-butanol (Fraction V, 2.33% wt. by wt.) and a water soluble portion (Fraction VI, 4.16% wt. by wt.). The exhausted plant material was assigned as Fraction VII (see also Scheme 2.1). The procedure for extraction and isolation is presented in Scheme 3.1.

Scheme 3.1 The extraction and isolation procedure for the leaves of R. apiculata Bl.



3.3 Chemical Constituents of Fraction I

Fraction I as a greenish sticky crude 229.10 g. (4.60% wt. by wt. of plant material) was obtained from extraction the leaves of R. apiculata with dichloromethane.

The results of separation and purification of crude Fraction I by means of column chromatography yielded fifteen crystalline compounds which were designated as Cpd.1 m.p. 66.0-66.5 °C (0.0092% wt.*), Cpd.2 m.p. 73.0-75.0 °C (0.1312 % wt.*), Cpd.3 m.p. 70.0-71.5 °C (0.0758% wt.*), Cpd.4 m.p. 218.0-219.5 °C (0.0012% wt.*), Cpd.5 m.p. 128.0-129.0 °C (0.0031% wt.*), Cpd.6 m.p. 83.0-85.0 °C (0.0016% wt.*), Cpd.7 m.p. 196.0-198.0 °C (0.0023% wt.*), Cpd.8 m.p. 213.0-215.0 °C (0.0406% wt.*), Cpd.9 m.p. 279.0-280.0 °C (0.0735% wt.**), Cpd.10 m.p. 143.0-145.0 °C (0.0085% wt.*), Cpd.11 m.p. 275.0 °C (dec.) (0.0008% wt.*), Cpd.12 m.p. 263.0 °C (dec.) (0.0032% wt.*), Cpd.13 m.p. 229.0-230.0 °C (0.0028% wt.*), Cpd.14 m.p. 260.0 °C (dec.) (0.0092% wt.*) and Cpd.A m.p. 285.0 °C (dec.) (0.0002% wt.*).

The major components of this fraction were Cpd.2, Cpd.3, Cpd.8 and Cpd.9.

---

* The percentage yield was calculated compared with dried plant material.

3.3.1 Structural Elucidation of Compound 1

Compound 1, m.p. 66.0-66.5 °C, was isolated from crude Fraction IA by silica gel column chromatography and recrystallized from ethylacetate for several times to yield Compound 1 as bright white plate 600 mg. (0.40% wt. by wt. of Fraction I). It showed only one spot at Rf 0.81 using 3% dichloromethane in n-hexane as a developing solvent. This compound gave negative results to Liebermann-Burchard's, $FeCl_3$, Br_2 in CCl_4 and 2,4-DNP reagents which indicated that it was not composed of steroidal or triterpenoidal skeleton, phenolic group, unsaturation part and carbonyl as functional groups, respectively.

The IR spectrum of Compound 1 (Fig.7) was similar to that of paraffin oil which was suggested that this substance should be a saturated long chain aliphatic hydrocarbon compound. The IR absorption band assignments of Compound 1 are given in Table 3.1 (66-67). This compound did not show any absorption peaks in ultraviolet region. This information revealed the absence of any functional groups as chromophores in Compound 1.

The mass spectrum of Compound 1 (Fig.8) displayed the molecular ion peak, M^+, at m/e (% rel int.) 464.0 (0.20). Other important ion peaks were observed at m/e 450.0 (0.60), 436.0 (6.10), 422.0 (1.40), 408.0 (5.30) and so on. This data indicated that this substance was lost $-CH_2-$ group (m/e 14) step by step (68). From the mass spectrum as well as the IR spectrum hinted that Compound 1 should be a saturated long chain aliphatic hydrocarbon, tritriacontane ($C_{33}H_{68}$: MW. 464.54).

Table 3.1 The IR absorption band assignments of Compound 1

Frequency (cm^{-1})	Band type	Tentative assignments
2960	s	C-H asym. stretch. vibration of CH_3-
2920	s	C-H asym. stretch. vibration of $-CH_2-$
2850	s	C-H sym. stretch. vibration of $-CH_2-$
1475	s	C-H asym. bend. vibration of CH_3-
1465	s	C-H asym. bend. vibration of $-CH_2-$
1375	w	C-H sym. bend. vibration of CH_3-
730,720	m	C-H rocking mode of $-CH_2-$ (for chains > 4 carbons)

However, The GLC analysis data (Fig.9) exhibited that Compound 1 was in fact a mixture of 7 saturated long chain aliphatic hydrocarbon by using well-known method to construct correlation standard curve between logarhithm of retention time and numbers of carbon atom in standard samples* (69). The correlation standard curve is presented in Fig.10.

From the GLC analysis of Compound 1 showed 7 peaks with retention time at 3.73, 4.76, 6.11, 7.83, 10.13, 12.93 and 16.60 min., respectively which corresponded to numbers of carbon

---

* Standard samples are hexacosane ($C_{26}H_{54}$), octacosane ($C_{28}H_{58}$), triacontane ($C_{30}H_{62}$) and dotriacontane ($C_{32}H_{66}$).

as 27, 28, 29, 30, 31, 32 and 33, respectively. Besides, from the gas chromatogram revealed that the peak with retention time 10.13 min. was the major component in this mixture.

Therefore, Compound 1 supported by physical properties and spectroscopic data was a mixture of 7 saturated long chain aliphatic hydrocarbon; heptacosane ($C_{27}H_{56}$), octacosane ($C_{28}H_{58}$), nonacosane ($C_{29}H_{60}$), triacontane ($C_{30}H_{62}$), hentriacontane ($C_{31}H_{64}$), dotriacontane ($C_{32}H_{66}$) and tritriacontane ($C_{33}H_{68}$). Hentriacontane ($C_{31}H_{64}$) was the major one, 52.83% of this mixture, while heptacosane ($C_{27}H_{56}$) and octacosane ($C_{28}H_{58}$) were present as trace components. The structure of Compound 1 is shown below:

$$CH_3\text{-}(CH_2)_n\text{-}CH_3 \qquad n = 25,26,\ldots,31$$

Compound 1

The composition of saturated long chain aliphatic hydrocarbons contained in Compound 1 is given in Table 3.2.

Table 3.2 The composition of saturated long chain aliphatic hydrocarbons contained in Compound 1

Name	Formular	% Composition
heptacosane	$CH_3-(CH_2)_{25}-CH_3$	0.34
octacosane	$CH_3-(CH_2)_{26}-CH_3$	0.76
nonacosane	$CH_3-(CH_2)_{27}-CH_3$	17.56
triacontane	$CH_3-(CH_2)_{28}-CH_3$	8.38
hentriacontane	$CH_3-(CH_2)_{29}-CH_3$	52.83
dotriacontane	$CH_3-(CH_2)_{30}-CH_3$	10.36
tritriacontane	$CH_3-(CH_2)_{31}-CH_3$	9.76

3.3.2 Structural Elucidation of Compound 2

Compound 2, one of the major components of Fraction I, was collected from the crude Fraction IA as well as Fraction IB by silica gel column chromatography. After recrystallization from a mixture of chloroform and acetone for several times, Compound 2 was obtained as white amorphous solid 8.53 g. (5.69% wt. by wt. of Fraction I), m.p. 73.0-75.0 °C, Rf 0.69 (solvent: 20% chloroform-hexane). This compound gave a violet colour with Liebermann-Burchard's and decolourized Br_2 in CCl_4 reagents which suggested that there should be a triterpenoidal skeleton with unsaturation part in its structure (55,60-61).

The IR spectrum of Compound 2 (Fig.11), which is assigned in Table 3.3 (66-67), indicated that this compound contained an ester functional group (at 1735 cm^{-1}). According to the

IR spectrum and colour tests, Compound 2 should be an unsaturated triterpenoidal ester.

Table 3.3 The IR absorption band assignments of Compound 2

Frequency (cm^{-1})	Band type	Tentative assignments
2950	s	C-H stretching vibration of CH_3-
2900,2850	s	C-H stretching vibration of $-CH_2-$
1735	s	$-\overset{O}{\overset{\|}{C}}-$ stretching vibration of ester
1650	very w	C=C stretching vibration
1475	m	C-H bending vibration of CH_3-
1380,1360	m	C-H bending vibration of gem-dimethyl
1170	m	C-O stretching vibration of propionates or higher
730,720	w	C-H rocking vibration of $-CH_2-$ (for chain > 4 carbons)

The UV ($CHCl_3$) spectrum (Fig.15) gave the maxima absorption peaks (λ max) at 203 nm. (log ε = 5.80) and 287 nm. (log ε = 3.99) which indicated that there should be a non-conjucated unsaturation and a carbonyl functional group as its chromophores (70).

The ^1H NMR spectrum (Fig.13) exhibited the triplet peak at δ 5.18 ppm. (J=3.51 Hz) which indicated an olefinic proton attached to a methylene group ($-CH_2-\underline{CH}=C\langle$) (71). Two signals belonged to protons involving to ester could be assigned. One which

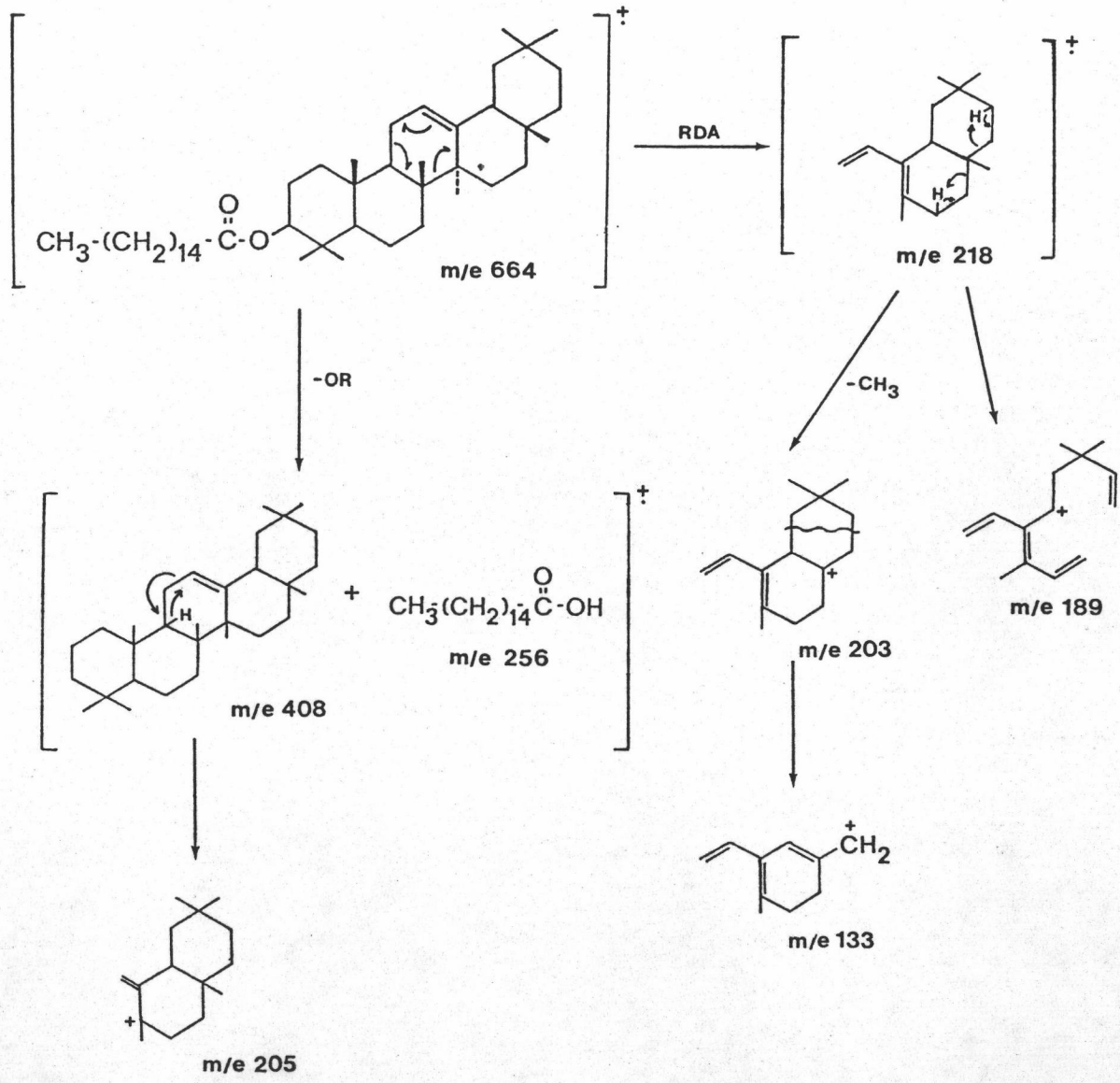
was a triplet signal at δ 4.52 ppm. (J=7.90 Hz) should be the chemical shift of α-proton in the alcoholic portion of the molecule ($R-\overset{O}{\overset{\|}{C}}-O-\overset{H}{\overset{|}{C}}-CH_2-$) and the other was also a triplet signal at δ 2.30 ppm. (J=7.03 Hz) which could be assigned for the chemical shift of α-proton in the acidic part ($-CH_2-\overset{H}{\overset{|}{C}}-\overset{O}{\overset{\|}{C}}-OR$) (63). Other signals around 1.88 to 0.86 ppm. ought to be the signals of methyl, methylene and methine protons (71).

The ^{13}C NMR spectrum (Fig.14) displayed the important signal at 173.63 ppm. which was corresponded to a carbon signal of ester. Other signals at 145.19 and 121.62 ppm. could be assigned for olefinic carbons. The ^{13}C NMR off resonance spectrum (Fig.14b) found that the signals at 173.63 and 145.19 ppm. still showed only one peak, whereas the signal at 121.62 ppm. was splitted to doublet. This information hinted that there was no hydrogen atom bonded to carbons positioned at 173.63 and 145.19 ppm. Thus, the carbon type at the position 173.63 ppm. should be $-\underline{\overset{O}{\overset{\|}{C}}}-O-$ and at 145.19 ppm. ought to be $R_1R_2\underline{C}=$, while there should be a skeleton like $\overset{H}{\diagdown}\!\!>C=$ at 121.62 ppm.. The signal at 80.50 ppm. could be assigned for an α-carbon which was attached to oxygen atom. In the ^{13}C NMR off resonance spectrum, this signal was splitted to doublet. Hence, this α-carbon must be only one hydrogen atom attaching to ($R-\overset{H}{\overset{|}{C}}-OH$). Other signals in both the ^{13}C NMR and the ^{13}C NMR off resonance spectra corresponded to the signals of methyl, methylene, methine and quarternary carbons. The signal at 26.96 ppm. which was analogous with methylene carbon was notified because of its high intensity. This information revealed that there ought to be several interlinking of methylene groups in the molecule of this compound.

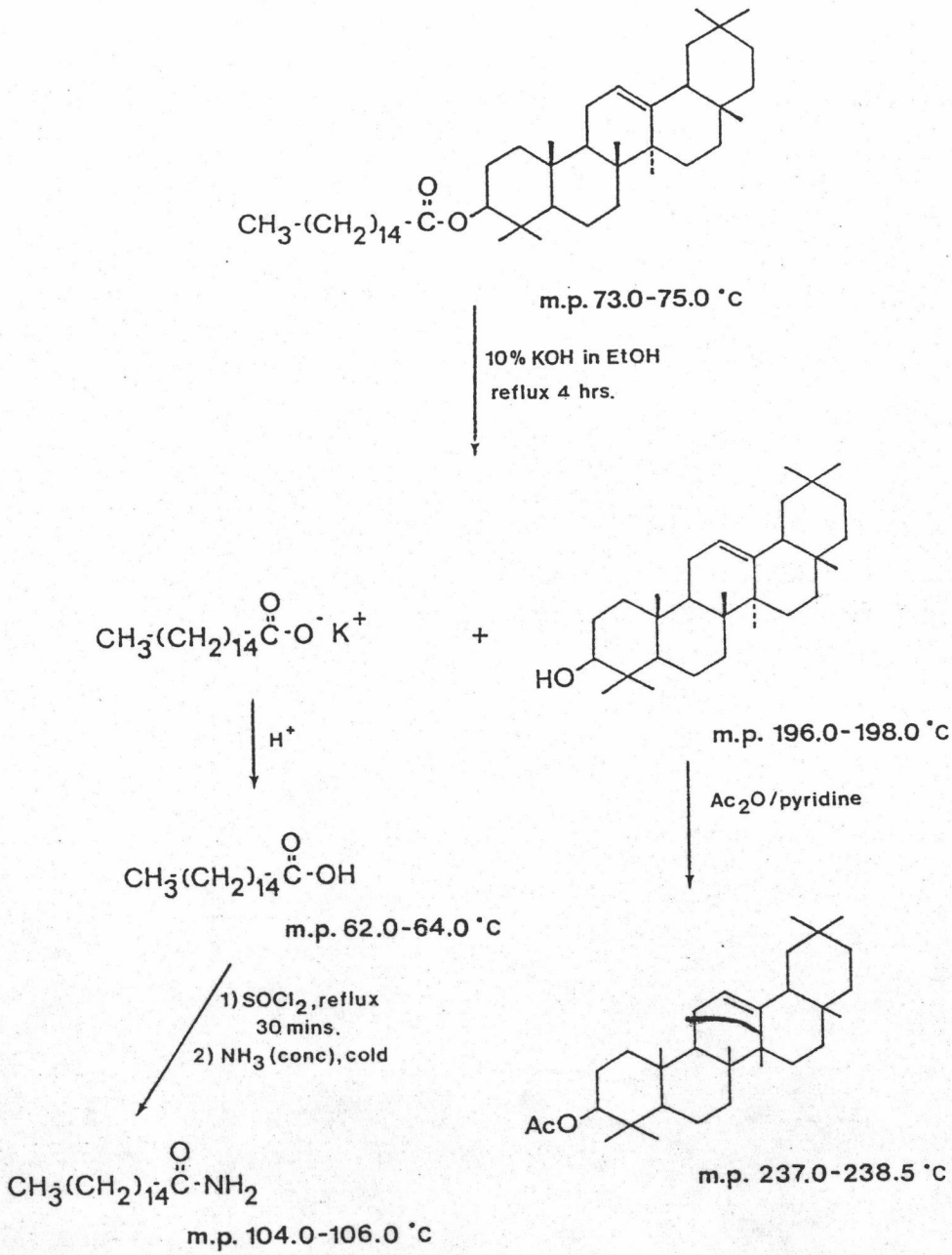
The mass spectrum (Fig.12) displayed the molecular ion peak at m/e (% rel int.) 664.0 (0.60, M^+) (Calcd. for $C_{46}H_{80}O_2$: MW. 664.63). The fragmentation ion peak at m/e 649.0 (0.20) was corresponded to cleave CH_3- (m/e 15) from the molecule. Other important peaks were observed at m/e 408.0 (32.70) which indicated that $C_{16}H_{32}O_2$ (m/e 256) was lost from the parent ion peak by alpha cleavage and at m/e 256.0 (47.80) which suggested to be lost $C_{30}H_{48}$ from the parent peak in usual fragmentation of ester compounds (63). Other important peaks were detected at m/e 393.0 (12.13), 218.0 (100.00, 408-RDA), 203.0 (37.00, 218-CH_3), 189.0 (25.00) and 133.0 (11.00). From the fragmentation ion pattern hinted that Compound 2 should belong to oleanane or ursane series (73) as well as a carboxylic part which may be palmitic acid ($C_{16}H_{32}O_2$: MW. 256).

The possible fragmentation pattern of Compound 2 is presented in Scheme 3.2 and the chemical reactions of Compound 2 are shown in Scheme 3.3.

Scheme 3.2 The possible mass fragmentation pattern of Compound 2



Scheme 3.3 The chemical reactions of Compound 2



Base hydrolysis of Compound 2 was set up in order to simplify structural elucidation of this compound. One of the hydrolyzed product, Compound 2A (an alcoholic part) as bright white feather crystal, m.p. 196.0-198.0 °C, Rf 0.37 (solvent: chloroform), gave a violet colour with Liebermann-Burchard's and also decolourized Br_2 in CCl_4 reagents. This results confirmed that the alcoholic part should be a triterpenoid compound with unsaturation part (55,60-61).

The IR spectrum of this triterpenoid (Fig.16) revealed the information which was agreed with colour tests: Br_2 in CCl_4, i.e., there was the absorption band belonging to C=C stretching vibration at ν_{max}^{KBr} 1645 cm^{-1}. Other important bands were observed at 3290 and 1040 cm^{-1} which were the characteristic of O-H stretching vibration and C-O stretching vibration, respectively (66-67).

The ^1H NMR spectrum of Compound 2A (Fig.18) exhibited the triplet signal at δ 5.19 ppm. (1H,J=3.50 Hz) which was an olefinic proton. From this data, there should be a skeleton like $-CH_2-C\underline{H}=C\langle$ in this molecule. At δ 3.23 ppm. displayed 1 H, multiplet, which could be assigned for the proton bearing hydroxy group ($-\underset{H}{\overset{|}{C}}-OH$) (71). Other signals around 1.89-0.71 ppm. in the ^1H NMR spectrum should be the signals of methyl, methylene and methine protons.

The mass spectrum (Fig.17) displayed the molecular ion peak at m/e (% rel. int.) 426.0 (9.90) (Calcd. for $C_{30}H_{50}O$: MW. 426.39). Other important fragmentation ion peaks were observed at m/e 218.0 (100.00), 207.0 (6.99) and 189.0 (8.79) corresponded to

the triterpenoids belonging to oleanane and ursane groups (73). Considering the molecular formular, spectroscopic information and type of triterpenoids found that this compound may be α-amyrin, β-amyrin, epi- β-amyrin or δ-amyrin. Fortunately, literature studies found that these four triterpenoids and their acetyl derivatives showed different melting points. The comparision of these four triterpenes is presented in Table 3.4.

Table 3.4 The comparision data of α-amyrin, β-amyrin, epi- β-amyrin and δ-amyrin (74)

Triterpenoids	m.p. (℃)	m.p. of acetyl derivatives (℃)
α-amyrin	186.0	225.0-226.0
β-amyrin	197.0-197.5	236.0-238.0
epi-β-amyrin	225.0	128.0
δ-amyrin	212.0-213.5	208.0-209.5
Compound 2A	196.0-198.0	237.0-238.5

From the comparision data, Compound 2A and its acetate were found to correspond to β-amyrin and β-amyrinacetate rather than other triterpenoids. Therefore, Compound 2A -an alcoholic part of Compound 2- should be β-amyrin.

Moreover, the ^{13}C NMR spectrum of Compound 2A could be used as the best information for ensuring that this compound was exactly β-amyrin. The chemical shifts of Compound 2A, Compound 2 and β-amyrin are presented in Table 3.5 (75-76).

Table 3.5 The ^{13}C NMR chemical shifts of β-amyrin, Compound 2A and Compound 2

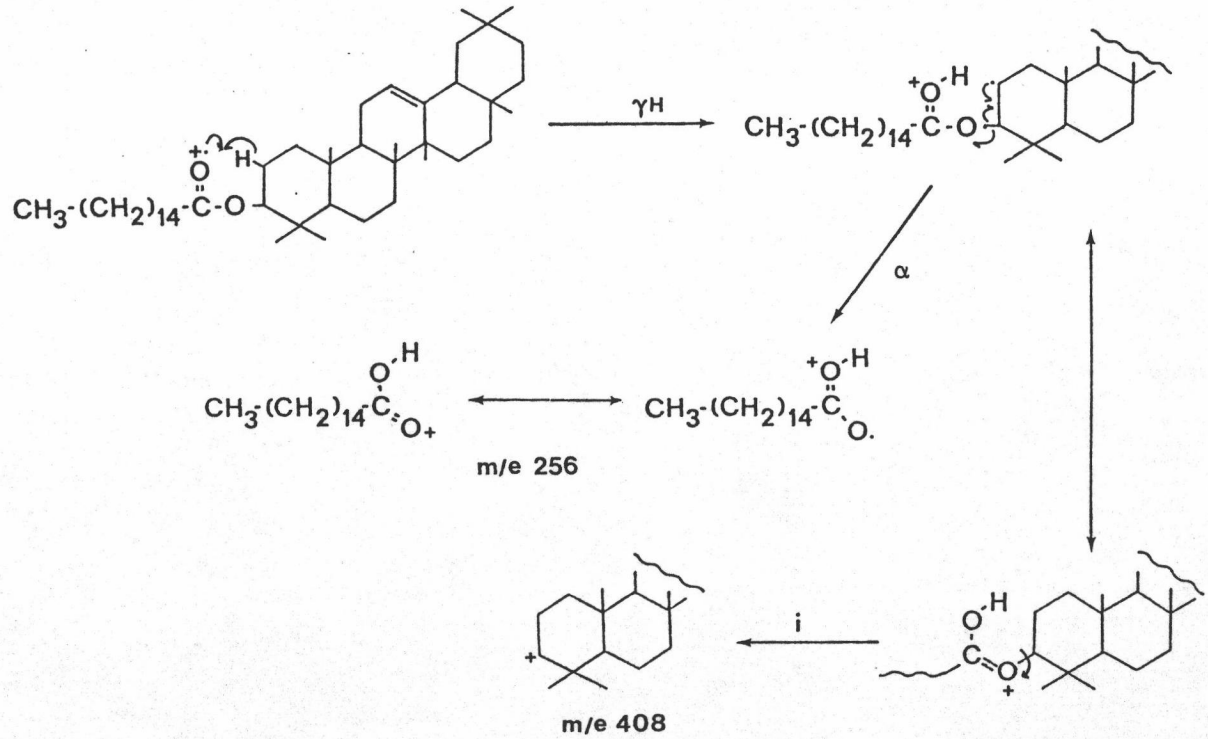
Carbon	Chemical shifts (ppm.)		
	β-amyrin	Compound 2A	Compound 2
1	38.5	38.60	37.70
2	27.0	26.98	26.92
3	78.9	79.04	80.50
4	38.7	38.79	38.19
5	55.1	55.20	55.20
6	18.3	18.42	18.26
7	32.6	32.67	32.56
8	39.7	39.82	39.76
9	47.6	47.67	47.51
10	37.0	37.00	37.11
11	23.4	23.57	23.67
12	121.7	121.73	121.62
13	145.0	145.19	145.13
14	41.7	41.77	41.66
15	28.3	28.44	28.39
16	26.2	26.17	26.11
17	32.5	32.50	32.45
18	47.2	47.24	47.18
19	46.8	46.86	46.75
20	31.1	31.10	31.04
21	34.8	34.78	34.83
22	37.2	37.16	36.78
23	28.1	28.12	28.01
24	15.5	15.60	15.55
25	15.5	15.60	15.55
26	16.8	16.85	16.74
27	26.0	26.00	25.95
28	27.3	27.25	26.92
29	33.2	33.37	33.32
30	23.6	23.73	23.56

* Compound 2 showed other peaks at δ (ppm.): 14.08(1C), 22.70(1C), 29.14(1C), 29.25(2C), 29.36(2C), 29.47(1C), 29.68(5C), 31.91(1C), 34.72(1C) and 173.63(1C).

After acidification and purification the other portion which was derived from the base hydrolysis of Compound 2, a white amorphous product, designated as Compound 2B, was obtained. The colour test of this compound suggested the absence of unsaturated triterpenoid or steroid.

The IR spectrum (Fig.21) informed that this amorphous product should contain a carboxyl as its functional group (ν_{max}^{KBr} 3600-3000 (O-H streth.), 1700 (-$\overset{O}{\overset{\|}{C}}$- of acid), 1430,1410,1310 and 1290 (C-O stretch. coupled with O-H bend.) and 935 (O-H bend.)) together with the absorption peaks at 2920,2850 (C-H stretch.), 1465 (C-H bend.) and 720 (C-H rock.) which indicated the presence of a saturated long chain aliphatic compound.

Considering the mass spectrum of Compound 2, the important fragmentation ion peaks, besides the parent ion peak, were observed at m/e 408.0 (32.70, M^+-256) and 256.0 (17.30, M^+-408). These two fragmentation ion peaks suggested that $C_{16}H_{32}O_2$ (palmitic acid, MW. 256) was lost from the parent compound by α- and β-cleavage with γ-hydrogen rearrangement in usual fragmentation of ester substances (53,68,77).



To confirm that Compound 2B was palmitic acid, the conversion of this compound to Compound 2B amide was performed and found that both their IR spectra and m.p. were corresponded to the reported palmitamide (74,78).

Moreover, this long chain carboxylic acid was confirmed further by using both the GLC and the HPLC analysis. The results revealed that Compound 2B was clearly to conclude to be palmitic acid.

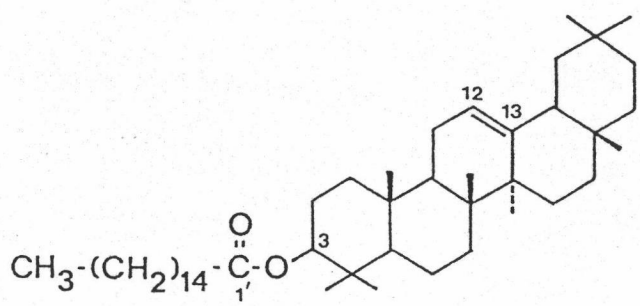
Supported by various methods, Compound 2B ,therefore, must be palmitic acid.

Reconsidering the ^{13}C NMR spectrum of Compound 2 found that this information was also agreed with β-amyrinpalmitate. Owing to the signals on the ^{13}C NMR spectrum, they were similar to β-amyrin (75,76). After substraction of the signals of β-amyrin, the rest ten signals at chemical shift δ (ppm.): 14.08(1C),

22.70(1C), 29.14(1C), 29.25(2C), 29.36(2C), 29.47(1C), 29.68(5C), 31.91(1C), 24.72(1C) and 173.63(1C) were corresponded to palmitic ester compounds (79).

Besides, the β-amyrinpalmitate was synthesized and found that the synthetic β-amyrinpalmitate and the isolated one were absolutely idenical.

Hence, it is no doubt to conclde that Compound 2 -one of the major products in the dichloromethane crude extract- is β -amyrinpalmitate. The structure of this substance is shown below:



Compound 2 β-Amyrinpalmitate

3.3.3 Structural Elucidation of Compound 3

Compound 3, m.p., 70.0-71.5 °C, 4.93 g. (3.29% wt. by wt. of Fraction I) was eluted from the column of Fraction IB with 5% chloroform-hexane in Fraction No. 9-15, recrystallized by a mixture of chloroform and acetone as white amorphous product. This compound did not show a single spot on TLC plate, but displayed two closely spots at Rf value 0.73 and 0.70 using 50% chloroform-hexane as a mobile phase. The former at Rf 0.73 was identical to Compound 2. Though the solvents for recrystallization were changed, the TLC results still revealed two closely spots. The attempts to separate these two compounds by both column chromatography and PTLC were unsuccessful. This compound gave a violet colour when being treated

with Liebermann-Burchard's and also decolourized Br_2 in CCl_4 reagents which hinted that this compound should contain triterpenoidal nucleus with unsaturation part (55, 60-61). The physical properties of this substance were nearly Compound 2, such as its solubility.

The IR spectrum of Compound 3 (Fig.26) gave the absorbtion peaks identical to those of Compound 2 (Fig.11) which informed that Compound 3 was an ester compound (ν_{max}^{KBr} 1730 cm^{-1}) and contained saturated interlinking methylene moiety (ν_{max}^{KBr} 2950-2850, 1465 and 720 cm^{-1}) (66-67).

The mass spectrum of this compound gave two patterns which were agreed with the results from TLC, i.e., it pointed out that this compound was not composed of a single component.

The mass spectrum pattern 1 (Fig.27) was absolutely coincided with that of Compound 2. To illustrate, it gave the molecular ion peak at m/e (% rel int.) 664.0 (0.38) (Calcd. for $C_{46}H_{80}O_2$: MW. 664.61) together with other fragmentation peaks at m/e 408.0 (2.18, M^+-256), 256.8 (0.59, M^+-408), 218.0 (100.00, 408-RDA), 203 (27.46, 218-CH_3) and 189.0 (16.04).

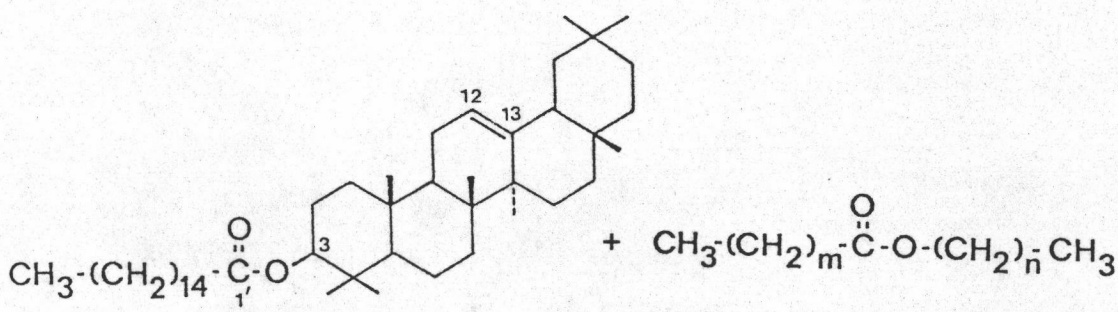
It was lucid to conclude that one of the components in this mixture was β-amyrinpalmitate, since the IR spectrum, the result from TLC (the upper spot) and the mass spectrum data of pattern 1 were identical to Compound 2.

The mass spectrum pattern 2 (Fig.28) gave the pattern of saturated hydrocarbon like, due to the fragmentataion of chopping -CH_2- (m/e 14) step by step. The base peak was observed at m/e 43.0 which always occured in saturated long chain compounds (68).

Unfortunately, the mass spectrum pattern 2 did not give the molecular ion peak. Thus, it could not assign the possibility of the molecular formular of this compound. However, from the IR and the mass spectrum evidences, this substance may be an ester of saturated long chain carboxylic acid and saturated long chain alcohol.

It is not surprising to isolate a mixture of β-amyrinpalmitate and other ester compounds, especially an ester of long chain compounds as a mixture. Since literatures have cited that β-amyrinpalmitate always occured and separated from the waxy fractions of the plant (74).

Nevertheless, the possible structure of Compound 3 could conclude to be a mixture of β-amyrinpalmitate and saturated long chain ester compound (a wax). Their structures can be drawn as follows:



Compound 3

3.3.4 Structural Elucidation of Compound 4

Compound 4 was separated from the silica gel column chromatography of Fraction IC by 10% chloroform-hexane. After recrystallization with a mixture of chloroform and methanol, white amorphous product 40 mg. (0.03% wt. by wt. of Fraction I), m.p. 218.0-219.0 °C, Rf 0.68 (solvent: chloroform) was obtained. This compound gave a purple colour to Liebermann-Burchard's and decolourized Br_2 in CCl_4 reagents.

The IR spectrum (Fig.29), which is assigned as shown in Table 3.6, revealed the strong absorption peaks at ν_{max}^{KBr} 1730 and 1265 cm^{-1} due to the $-\overset{O}{\overset{\|}{C}}-$ and C-O stretching vibration of ester, respectively. The information obtained from both the colour tests and the IR spectrum hinted that Compound 4 should be an unsaturated triterpenoidal ester.

Table 3.6 The IR absorption band assignments of Compound 4

Frequency (cm^{-1})	Band type	Tentative assignments
3050	very w	C-H stretching vibration of $R_1R_2C{=}CHR_3$
2950,2850	s	C-H stretching vibration of CH_3-, $-CH_2-$
1730	s	$-\overset{O}{\overset{\|}{C}}-$ stretching vibration of ester
1480,1380	w	C-H bending vibration of gem-dimethyl groups
1265	w	C-O stretching vibration of ester

The mass spectrum (Fig.30) gave the molecular ion peak at m/e (% rel int.) 678.0 (Calcd. for $C_{47}H_{82}O_2$: MW. 678.64). Other fragmentation ion peaks were observed at 660.0 (78.23), 422.0 (23.00), 269.0 (100.00), 256.0 (19.68), 204.0 (63.70) and 181.0 (24.20). Like Compound 2, two significant fragmentation ion peaks were observed at m/e 422.0 (M^+-256) and 256.0 (M^+-422) which implied that the acidic part in this compound may be palmitic acid.

The ^1H NMR spectrum (Fig.31) revealed the double doublet signal at 5.52 ppm. (1H,J=2.50,7.51 Hz) compatible with an olefinic proton. The triplet signal with 1H integration centered at 4.53 ppm. was ascribed for the α-proton in the alcoholic portion of the molecule (R-C(=O)-O-C(H)-CH_2-). The triplet signal at 3.10 ppm. and the doublet signal at 2.79 ppm. (1H each) were concided with the structure like >CH-CH_2- and >CH-CH< ,respectively. The singlet signal at 1.26 ppm. was analogous to methylene interlinking system in the molecule. Eight methyl signals were observed at 1.10, 1.00, 0.96, 0.90, 0.82 3H each) and 0.86 (6H) ppm..

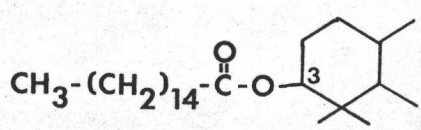
The ^{13}C NMR spectrum (Fig.32) exhibited the carbonyl of ester at 173.52 ppm. and two olefinic carbons at 157.00 and 118.90 ppm.. The signal at 80.29 ppm. could be assigned for the carbon adjacent to oxygen of ester linkage. Other signals were concided with methyl, methylene, methine and quarternary carbons. The high intensity singlet signal at 29.69 ppm. was corresponded to the presence of methylene interlinking system in this compound.

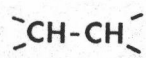
The UV (EtOH) spectrum (Fig.33) gave the maximum absorption peak at 206 nm. (log ε = 5.48) which was pointed out non-conjugated unsaturation chromophore.

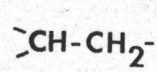
Basic hydrolysis of Compound 4 was set up and yielded Compound 4A which was designated for the alcoholic portion and Compound 4B which was labelled for the acidic one. The latter was proved to be palmitic acid by the GLC analysis.

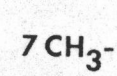
According to the colour tests and the IR spectrum, Compound 4A was found to be an unsaturated triterpenoid. The mass spectrum of this compound gave the molecular ion peak at 440.0 (Calcd. for $C_{31}H_{52}O$: MW. 440.41). Hence, this compound ought to be a triterpenoid $C_{31}H_{52}O$ joined to palmitic acid by ester linkage.

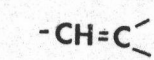
This compound was obtained in such a small amount that its structure was not definitely established, however, some possible parts of this substance are shown as follows:











3.3.5 Structural Elucidation of Compound 5

Compound 5 was isolated from the separation Fraction IC with 20% chloroform-hexane. After fractional recrystallization by using a mixture of chloroform and methanol, Compound 5 as bright white plate 200 mg. (0.13% wt. by wt. of Fraction I), m.p. 128.0-129.0 °C, Rf 0.68 (solvent: chloroform), was gained. This compound gave a violet colour with Liebermann-Burchard's, decolourized Br_2 in CCl_4 and also gave a positive test to 2,4-DNP reagents which represented the triterpenoidal skeleton, unsaturation part and carbonyl functional group in its structure (55,60-61).

The IR spectrum (Fig.40), which could be assigned as given in Table 3.7, gave good agreements to colour tests. Its IR spectrum revealed the absorption bands due to α -, β-unsaturated ketone at 1650 and 1620 cm^{-1}. The peaks which were corresponded to $-\overset{O}{\overset{\|}{C}}-$ stretching vibration of ester and C-H bending vibration of gem-dimethyl group were observed at 1730 and 1385, 1360 cm^{-1}, respectively. From both information, Compound 5 was suggestive of being α, β -unsaturated ketone triterpenoidal ester (66,67).

The presence of α,β-unsaturated ketone was supported by the UV spectrum. The UV spectrum of Compound 5 (Fig.44) gave the λ max at 248 nm. ($\log \varepsilon = 5.54$) which was corresponded to that of the calculation for α, β -unsaturated ketone chromophore (70).

Table 3.7 The IR absorption band assignments of Compound 5

Frequency (cm^{-1})	Band type	Tentative assignments
2900,2850	s	C-H stretching vibration of CH_3-, $-CH_2$-
1730	s	$-\overset{O}{\overset{\|}{C}}-$ stretching vibration of ester
1650	s	$-\overset{O}{\overset{\|}{C}}-$ stretching vibration of α,β-unsaturated ketone
1620	w	C=C stretching vibration
1470,1450	m	C-H bending vibration of CH_3-, $-CH_2$-
1385,1360	m	C-H bending vibration of gem dimethyl group
1205	m	C-O stretching vibration

The ^1H NMR spectrum (Fig. 42) displayed a singlet signal at 5.59 ppm. (1H) compatible with the presence of a $-\overset{|}{\underset{|}{C}}-\underline{CH}=C$ group (80). The two significant signals at 4.55 ppm. (1H,t,J=7.56 Hz) attributed to α-hydrogen in the alcoholic portion of ester molecule ($R-\overset{O}{\overset{\|}{C}}-O-\overset{|}{\underset{H}{C}}-CH_2-$) and at 2.36 ppm. (t,1H,J=6.36 Hz) characterized α-proton in the acidic portion ($-CH_2-\overset{|}{\underset{H}{C}}-\overset{O}{\overset{\|}{C}}-OR$) (63). Other signals around 1.93 to 0.88 ppm. should be the signals of methyl, methylene and methine protons. The high intensity peak at 1.26 ppm. indicated that this compound should be composed of a long chain methylene interlinking system in its structure (71).

The ^{13}C NMR spectrum (Fig.43) exhibited 37 carbon signals which could be assigned as follow: the signal at 200.12 ppm.

was fixed for a carbonyl carbon of ketone and the signal at 173.68 ppm. was suggestive of a carbon of ester ($R-\underset{\underset{O}{\|}}{C}-OR'$). The unusual carbon signals were detected at 170.60 and 128.07 ppm. which occured at higher field than other olefinic carbons. This information suggested that the olefinic part in this molecule should attach to a group which made a deshielding effect to both of them. From the ^{13}C NMR off resonance spectrum (Fig.43b), the chemical shift at 200.12, 173.68 and 170.60 ppm. revealed only one peak, whereas the signal at 128.07 ppm. was splitted to doublet. This result hinted that there was no hydrogen atom attached on the former carbons, while the structure like $\rangle C=CH-R$ should be existent at the latter one. It was strongly corresponded to the ^{1}H NMR spectrum that some part of this compound should be $-\underset{\underset{O}{\|}}{C}-CH=C\langle$. The signal at 80.29 ppm. was compatible with an α-carbon which was bonded to oxygen atom of ester linkage. At this position in the ^{13}C NMR off resonance spectrum, the signal was also splitted to doublet. This α-carbon, therefore, had to be a skeleton like $R-\underset{\underset{H}{|}}{C}-O-$. Other signals around 61.70 to 14.14 ppm. should be the signals of methyl, methylene, methine and quarternary carbons. The signal at 29.69 ppm. which was analogous with a methylene carbon was notified owing to its high intensity. This data suggested that there ought to be several interlinking of methylene groups in the molecule of this compound (72).

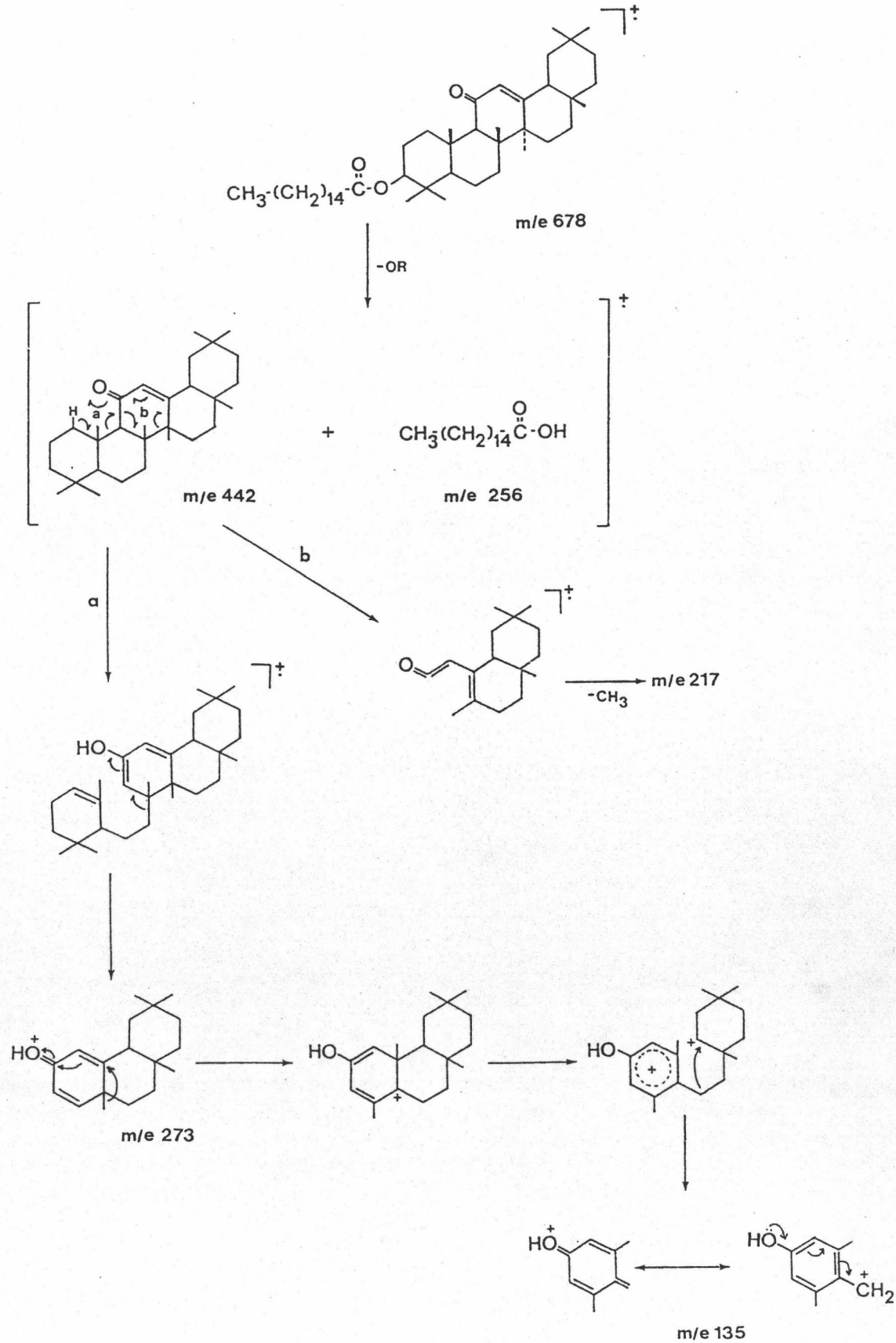
The information above supported to each other that Compound 5 had to contain α , β-unsaturated ketone and ester functional group in this triterpenoidal skeleton. The portion derived from carboxylic acid part should be composed of a several

methylene interlinking system.

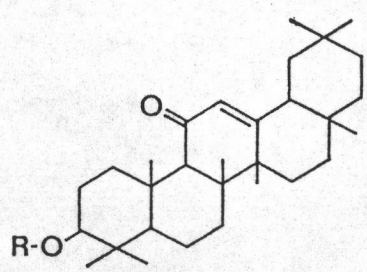
Compound 5 was proposed to be $C_{46}H_{78}O_3$ according to the mass spectrum data and the elemental analysis result.

The mass spectrum of this compound showed the parent ion peak at m/e (% rel int.) 678.0 (8.75) (Calcd. for $C_{46}H_{78}O_3$: MW. 678.61) together with two significant peaks at m/e 422.0 (41.60, M^+-$C_{16}H_{32}O_2$) and 256.0 (17.83, M^+-422). Like Compound 2, the presence of these two peaks suggested the acidic part may be palmitic acid (68,81). Other fragmentation ion peaks were observed at m/e 273.0 (69.94), 232.0 (100.00, RDA), 217.0 (15.60, RDA-CH_3) and m/e 135.0 (53.84). The possible mass fragmentation pattern is shown in Scheme 3.4 (80,82).

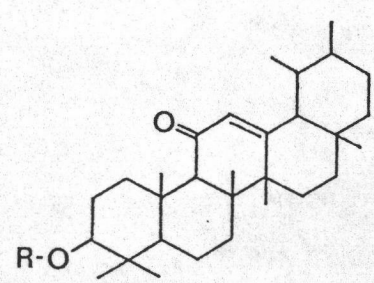
Scheme 3.4 The possible mass fragmentation pattern of Compound 5



There were some alternatives to place the functional group of $R-\overset{O}{\overset{\|}{C}}-CH=C\diagup$ in the skeleton of this pentacyclic triterpenoidal system. However, the most important characteristic fragmentation in unsaturated triterpenoid that always leads to the highest stable fragmentation ion species (base peak) is due to retro-Diels-Alder (RDA) fragmentation (73). This essential characteristic, hence, can be used to determine the right position of this functional group. According to a RDA fragmentation of Compound 5, it is infact that Compound 5 had a possibility to be either an olean-12-ene skeleton or an ursan-12-ene skeleton (73). Nevertheless, it was obviously to place the $R-\overset{O}{\overset{\|}{C}}-CH=C$ as either 11-oxo-olean-12-ene or 11-oxo-ursan-12-ene triterpenoid for Compound 5. Two possible structures of this compound as 11-oxo-olean-12-ene-3β - palmitate (β-amyrenonylpalmitate) (I) and 11-oxo-ursan-12-ene-3β - palmitate (α-amyrenonylpalmitate) (II) are drawn below:



(I)



(II)

(I),(II) R = $CH_3-(CH_2)_{14}-CO-$

Literature surveys on these two triterpenoids and their related compounds found that a mixture of α-amyrenonyl palmitate and β-amyrenonylpalmitate in ratio 1:1 was first isolated from the leaves of Carphephorus odoratissimus (80) and there was no report on both of them furthermore. However, there have been some publications involving to β-amyrenonol (I, R'=H) and α-amyrenonol (II, R'=H) in this recent years (83,84). Though the m.p. of a mixture of α- and β-amyrenonylpalmitate was not given in that report, those of their alcohols (α-amyrenonol and β-amyrenonol) were mentioned in other chemical literatures. Hence, it was essential to settle up the hydrolysis reaction of Compound 5 in order to prove that the alcoholic part of Compound 5 was infact either α-amyrenonol or β-amyrenonol. The basic hydrolysis reaction of Compound 5 was carried out by using 10% KOH in ethanol to afford Compound 5A as white needle crystal which melted at 229.0-230.5 °C, and Compound 5B which was identified as palmitic acid ($C_{16}H_{32}O_2$) by the GLC analysis. The IR spectrum of Compound 5A (Fig.45) was found to be coincided with those of the reported α-amyrenonol and β-amyrenonol (81,83,85-86), since these two triterpenoids gave the same characteristic absorption bands. Owing to a lack of the authentic samples, the direct comparision by Co-TLC and mixed m.p. is impossible. Although, these two interested triterpenoids gave the closely spectroscopic information, such as the IR and the mass spectra, the melting points of them and their derivatives, fortunately, were different. The comparision of melting point data among α-amyrenonol, β-amyrenonol and Compound 5 is given in Table 3.8.

Table 3.8 The comparision data among α-amyrenonol, β-amyrenonol and Compound 5

Compound	Melting point (°C)			Ref.
	Alcohol	Acetate	Palmitate	
α-amyrenonol	206-208	286-289	-*	83,85
β-amyrenonol	231-233	260-263	-*	84,86
Compound 5	229-230.5 (Cpd.5A)	-**	128-129	-

* The melting point of a mixture of α- and β-amyrenonylpalmitate was not reported

** Unsufficient of sample to prepare acetate derivative of Compound 5

From Table 3.8, it was no doubt to conclude that the alcoholic part of Compound 5 was β-amyrenonol.

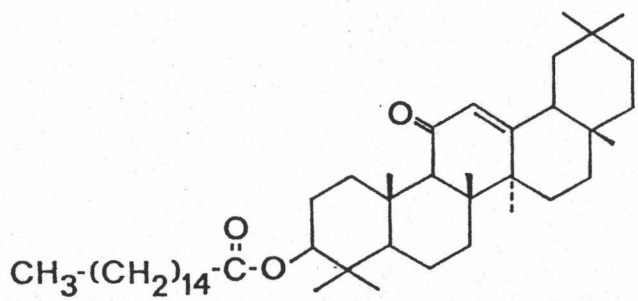
Throughout the chemical literatures, the ^{13}C NMR chemical shift assignments of β-amyrenonylpalmitate has not been reported, the tentative assignments of carbon chemical shifts of this compound are proposed as presented in Table 3.9 by comparing to those of the reported β-amyrenonylacetate.

Table 3.9 The ^{13}C NMR chemical shift assignments of Compound 5

Carbon	Chemical shifts (ppm.)	
	11-oxo-β-amyrenyl-acetate	Compound 5
1	38.7	38.79
2	23.4	23.40
3	80.5	80.28
4	38.0	38.08
5	54.9	55.04
6	18.4	18.74
7	32.6	32.72
8	43.3	43.39
9	61.5	61.70
10	36.9	36.95
11	201.4	200.12
12	127.9	128.07
13	170.2	170.60
14	45.3	45.45
15	26.4	26.44
16	26.4	26.44
17	32.3	32.40
18	47.5	45.45
19	45.0	45.18
20	31.0	31.04
21	34.4	34.45
22	36.4	36.51
23	28.0	28.06
24	16.6	16.79
25	15.7	16.41
26	17.3	17.39
27	23.5	23.51
28	28.7	28.77
29	33.0	33.05
30	23.6	23.51
$-\underline{C}O_2-$	170.6	173.68
$\underline{C}H_3-CO_2-$	21.2	-

* Compound 5 also showed other signals at δ (ppm.): 14.14(1C), 22.70(1C), 23.40(1C), 23.62(1C), 25.19(1C), 29.36(2C), 29.69(6C), 31.96(1C) and 34.83(1C).

Supported by various spectroscopic data, colour tests and chemical reactions, this compound, therefore, could be deduced the structure to be β-amyrenonylpalmitate. The structure of Compound 5 is shown below:



Compound 5 ß-Amyrenonylpalmitate

3.3.6 Structural Elucidation of Compound 6

Compound 6, m.p. 83.0-86.0 °C, 105 mg. (0.07% wt. by wt. of Fraction I) was eluted from the silica gel column of Fraction IC with 20% chloroform-hexane in Fraction No. 21 to No. 28. After recrystallization from hot n-hexane for several times, Compound 6 as white amorphous product was obtained. It showed a single spot on TLC plate at Rf 0.60 using chloroform as a developing solvent. This compound gave a negative test to Liebermann-Burchard's and could not change the colour of Br_2 in CCl_4 reagents. This result suggested that there were neither triterpenoidal, steroidal nor unsaturation part in its molecule (55,60-61).

The IR spectrum (Fig.48) exhibited the absorption peak at 3350 cm^{-1} indicated that Compound 6 should contain OH as its functional group. This information was supported by the absence of

this absorption peak when Compound 6 was acetylated (Fig.54). The characteristic peak at 1050 cm^{-1} was important to state C-O stretching vibration of primary alcohol ($R-CH_2-OH$). Other absorption bands were similar to Compound 1. Thus, this compound ought to be a saturated long chain aliphatic primary alcohol. The IR absorption band assignments of this compound are shown in Table 3.10 (66,67).

Table 3.10 The IR absorption band assignments of Compound 6

Frequency (cm^{-1})	Band type	Tentative assignments
3350	b,s	O-H stretching vibration
2930,2850	s	C-H stretching vibration of CH_3-, $-CH_2-$
1480,1470	m	C-H bending vibration of CH_3-, $-CH_2-$
1050	m	C-O stretching vibration of 1° ROH
730,720	w	C-H rocking mode of $-CH_2-$ (for carbon > 4)

Compound 6 did not show any absorption peaks in ultraviolet region, this information implied the absence of functional groups as chromophores in this substance.

The ^1H NMR spectrum (Fig.49) displayed the important triplet signal at 3.66 ppm. (2H) which should be the signal of the proton on the carbon attaching to oxygen atom. The presence of high intensity singlet signal at 1.26 ppm. revealed that there were several interlinking of methylene groups in the molecule of this compound. The singlet signal at 0.89 ppm. (3H) was corresponded to the methyl proton signal.

According to the colour tests, the IR and the ^{1}H NMR spectra, Compound 6 should be a saturated long chain aliphatic primary alcohol.

The ^{13}C NMR spectrum (Fig.50) displayed the signal of one carbon at 63.10 ppm. which was the carbon adjacent to OH. The signals at chemical shift around 32.85 to 22.71 ppm. referred to the chemical shifts of methylene carbons. The rest signal at 14.09 ppm. was assigned for the methyl carbon (72).

The molecular formular of Compound 6 was purposed to be $C_{34}H_{70}O$. This formular was supported by the mass spectrum data. In mass spectrum of a saturated aliphatic alcohol which contains many carbon atoms, the molecular ion peak, M^+, is usually very weak or missing. However, it always shows the prominent peak corresponded to the successive lose of one molecule of water from the molecular ion peak (M^+-18) together with the elimination of an olefin (68,71). Therefore, the fragmentation pattern of a primary alcohol is M^+ (sometime missing), M^+-H_2O, M^+-H_2O-olefins, i.e., (M^+-18), (M^+-46), (M^+-74), (M^+-102) and so on. In mass spectrum of Compound 6, the molecular ion peak was missing and there was the prominent one at m/e (% rel. int.) 476.0 (3.24) which should be M^+-H_2O peak. Other peaks were corresponded to the fragmentation pattern of a saturated primary alcohol, there were at 448.0 (12.81, $M^+-H_2O-(CH_2)_2$, M^+-46), 420.0 (14.28, 448.0-$(CH_2)_2$), 392.0 (4.87, 420.0-$(CH_2)_2$), 364.0 (2.12, 392.0-$(CH_2)_2$), 336.0 (2.07, 364.0-$(CH_2)_2$), 167.0 (11.51, 336.0-$(CH_2)_{11}-CH_3$) and 97.0 (167.0-$(CH_2)_5$). This information revealed that the molecular structure of this compound was composed of one methyl group, thirty three methylene groups and a hydroxyl

functional group. From the above spectral information, Compound 6 should be a saturated long chain primary alcohol -tetratriacontanol- ($C_{34}H_{70}O$).

Nevertheless, the GLC analysis data disclosed that Compound 6 was not a single compound, but it was a mixture of 7 saturated long chain aliphatic primary alcohol by using the plotted standard correlation curve between logarithm of retention time and the number of carbons in the authentic primary long chain alcohol samples* (69). The GLC analysis result and the standard correlation curve revealed 7 peaks on gas chromatogram (Fig.52 and 53) at retention time 7.71, 9.59, 11.75, 14.78, 18.28, 22.94 and 28.54 min., respectively which were corresponded to numbers of carbon 30, 31, 32, 33, 34, 35 and 36, respectively. The substances at Rt 11.75 and 18.28 min. were the major components in this mixture.

Hence, Compound 6 is infact a mixture of 7 saturated long chain aliphatic primary alcohol; triacontanol ($C_{30}H_{61}OH$), hentriacontanol ($C_{31}H_{63}OH$), dotriacontanol ($C_{32}H_{65}OH$), tritriacontanol ($C_{33}H_{67}OH$), tetratriacontanol ($C_{34}H_{69}OH$), pentatriacontanol ($C_{35}H_{71}OH$) and hexatriacontanol ($C_{36}H_{73}OH$), respectively. Dotriacontanol ($C_{32}H_{65}OH$) and tetratriacontanol ($C_{34}H_{69}OH$) are the major components in this mixture. The structure of Compound 6 is shown below:

---

* Standard samples are tetradecanol ($C_{14}H_{29}OH$), hexadecanol ($C_{16}H_{33}OH$), octadecanol ($C_{18}H_{37}OH$), icosanol ($C_{20}H_{41}OH$) and doicosanol ($C_{22}H_{45}OH$).

$$CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}OH \qquad n = 28,29,\ldots,34$$

Compound 6

The composition of saturated long chain aliphatic primary alcohols contained in Compound 6 is presented in Table 3.11.

Table 3.11 The composition of saturated long chain aliphatic primary alcohols contained in Compound 6

Name	Formular	% Composition
tritriacontanol	$CH_3\text{-}(CH_2)_{28}CH_2\text{-}OH$	0.73
hentriacontanol	$CH_3\text{-}(CH_2)_{29}CH_2\text{-}OH$	0.47
dotriacontanol	$CH_3\text{-}(CH_2)_{30}CH_2\text{-}OH$	32.28
tritriacontanol	$CH_3\text{-}(CH_2)_{31}CH_2\text{-}OH$	3.76
tetratriacontanol	$CH_3\text{-}(CH_2)_{32}CH_2\text{-}OH$	55.53
pentatriacontanol	$CH_3\text{-}(CH_2)_{33}CH_2\text{-}OH$	2.98
hexatriacontanol	$CH_3\text{-}(CH_2)_{34}CH_2\text{-}OH$	4.24

3.3.7 Structural Elucidation of Compound 7

Compound 7, m.p. 196.0-198.0 °C, 150 mg. (0.10% wt. by wt. of Fraction I) was collected from the silica gel column chromatography of Fraction IC in Fraction No. 8-14. This compound showed merely one spot at Rf 0.37 using chloroform as a solvent system. Compound 7 gave a violet colour when being treated with Liebermann-Burchard's and also decolourized Br_2 in CCl_4 reagents.

This information was indicative of triterpenoidal skeleton and unsaturation part in its structure, respectively (55,60-61).

The IR spectrum of Compound 7 could be assigned as shown in Table 3.12 (66-67).

Table 3.12 The IR absorption band assignments of Compound 7

Frequency (cm^{-1})	Band type	Tentative assignments
3290	b	O-H stretching vibration
2950,2920, 2850	s	C-H stretching vibration of CH_3-, -CH_2-
1645	w	C=C stretching vibration
1460	m	C-H bending vibration of CH_3-, -CH_2-
1380,1360	m	C-H bending vibration of gem-dimethyl gr.
1040,1020	m	C-O stretching vibration (probably 3β-OH)
810	w	C-H out of plane of trisub. or tetrasub. vinyl group

The information obtained from the colour tests and the IR spectrum revealed that this compound should be an unsaturated triterpenoidal compound which had a hydroxy group as its functional group. In triterpene, the absorption bands belonging to O-H and C-O stretching vibration could be used to indicate that the configuration at A/B ring should be 3α(OH) or 3β(OH) (87). The information is shown as follow

Configuration	O-H*	C-O
3 β-OH (e)	3630	1020 and 1040
3 α-OH (a)	3638	1065

* for monomeric O-H stretching vibration

Compound 7 showed the absorption peaks for hydrogen bonded O-H stretch. at 3290 cm^{-1} and for C-O stretch. at 1040 and 1020 cm^{-1} which were corresponded to 3β-OH rather than 3α-OH.

The UV spectrum (Fig.59) gave the λ max at 203 nm. (log ε = 3.75), this data showed the presence of non-conjugated system in this substance.

The ^1H NMR spectrum (Fig.57) displayed the proton signals in agreement with the information from the IR spectrum, i.e., there was an olefinic proton at 5.19 ppm. (1H,t,J=3.18 Hz). Another triplet signal centered at 3.23 ppm. (1H,t,J=7.69 Hz) was compatible with a proton bearing on carbon atom which was adjacent to hydroxy group. This signal can be also used to determine the configuration in A/B ring of triterpene. In six-member ring with chair conformation, the α-protons can be distinguished by their coupling constant (J), since J_{aa} (8-13 Hz) differs substantially from J_{ee} (1-5 Hz) (88). This above data strongly indicated that this compound was a triterpenoid containing 3β -OH. The ^1H NMR spectrum of this compound also exhibited eight methyl groups at δ (ppm.): 0.80(3H), 0.83(3H), 0.88(6H), 0.94(3H), 0.98(3H), 1.00(3H) and 1.14(3H). The ^1H NMR chemical shift assignments of Compound 7

was tabulated in Table 3.13 (89,90).

Table 3.13 The ^1H NMR chemical shift assignments of Compound 7

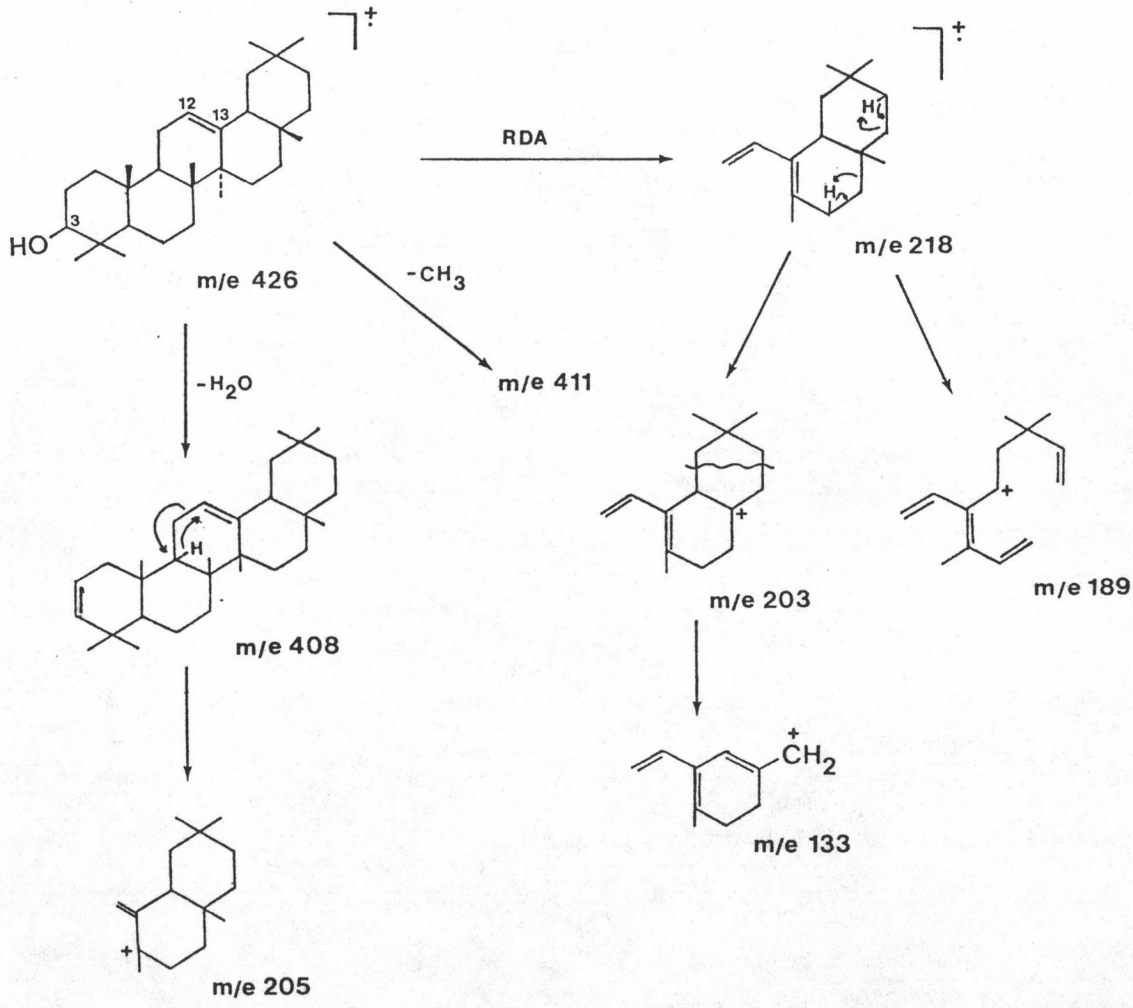
Compound	Chemical shift (ppm.)		
	olefinic proton	carbinol proton	methyl protons
β-amyrin	5.18	3.22	0.84(3H), 0.88(12H), 0.96-0.98(6H), 1.14 (3H)
Compound 7	5.19(1H,t, J=3.18 Hz)	3.23(1H,t, J=7.69 Hz)	0.80(3H), 0.83(3H), 0.88(6H), 0.94(3H), 0.98(3H), 1.00(3H), 1.14(3H)

The molecular formular of Compound 7 was purposed to be $C_{30}H_{50}O$ (MW. 426.39). This formular was supported by the mass spectrum data as well as the elemental analysis result.

The mass spectrum (Fig.56) gave the parent ion peak, M^+, at 426.0 (Calcd. for $C_{30}H_{50}O$: MW.426.39). The important fragmentation pattern at m/e (% rel. int.) 218.0 (100.00), 207.0 (7.10) and 189.0 (11.78) strongly pointed out that Compound 7 was a member of α-amyrin or β-amyrin series (73). These two series are characterized by the presence of a 12-13 double bond. This feature has proved to be readily recognizable by mass spectrometer, since the molecular ion undergoes the equivalent of a RDA fragmentation to furnish a very characteristic base peak m/e 218 in case of the absence of substituents constituted additional to the basic skeleton. The mass fragmentation pattern of Compound 7 is

presented in Scheme 3.5 (91).

Scheme 3.5 The possible mass fragmentation pattern of Compound 7



These two triterpenoids, α-amyrin and β-amyrin, gave closly characteristic peaks on mass spectrum, however, their m.p. and the signals on the ^{13}C NMR spectrum were different. The melting point comparision of α-amyrin, β-amyrin and their related compounds is shown in Table 3.4.

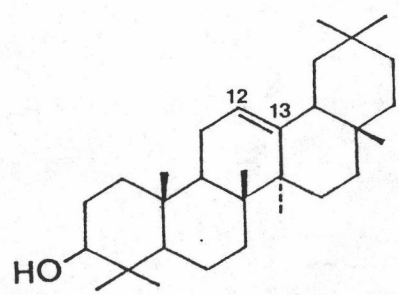
The ^{13}C NMR spectrum of this substance (Fig.58) displayed two olefinic carbons at 145.13 and 121.73 ppm. The former signal was proved from the ^{13}C NMR off resonance spectrum (Fig.58b) to contain a structure like $R_1R_2\text{-}\underline{C}\text{=}\dot{C}\text{-}$, while the latter was $R_1R_2C\text{=}\underline{C}H\text{-}$. The signal at 78.98 ppm. should be the carbon signal adjacent to oxygen atom bearing one proton ($R_1R_2\underline{C}H\text{-}OH$). Other signals around 55.14 to 15.45 ppm. ought to be methyl, methylene, methine and quarternary carbons which are assigned in Table 3.14.

Table 3.14 The ^{13}C chemical shift assignments of α-amyrin, β-amyrin and Compound 7

Carbon	Chemical shifts (ppm.)		
	α-amyrin	β-amyrin	Compound 7
1	38.7	38.5	38.57
2	27.2	27.0	26.92
3	78.8	78.9	78.98
4	38.7	38.7	38.73
5	55.2	55.1	55.14
6	18.3	18.3	18.36
7	32.9	32.6	32.66
8	40.0	39.7	39.64
9	47.7	47.6	47.61
10	36.9	37.0	36.94
11	17.4	23.4	23.51
12	124.3	121.7	121.73
13	139.3	145.0	145.13
14	42.0	41.7	41.71
15	28.7	28.3	28.38
16	26.6	26.2	26.16
17	33.7	32.5	32.45
18	58.9	47.2	47.24
19	39.6	46.8	46.85
20	39.6	31.1	31.04
21	31.2	34.8	34.72
22	41.5	37.2	37.10
23	28.1	28.1	28.06
24	15.6	15.5	15.45
25	15.6	15.5	15.45
26	16.8	16.8	16.79
27	23.3	26.0	25.94
28	28.1	27.3	27.19
29	23.3	33.2	33.31
30	21.3	23.6	23.67

To confirm this compound, the mixed m.p., Co. TLC, and comparision the spectral data of Compound 7 and Compound 2A which was proved to be β-amyrin, was performed and found that these two compounds were identical. Moreover, the acetyl derivative of Compound 7 was prepared and compared with the authentic β-amyrin acetate sample*, the results from mixed m.p., Co.TLC and comparision the retention time (Rt) from the GLC analysis were no doubt to conclude that this compound was β-amyrinacetate.

Supported by spectroscopic data, chemical reactions and comparision with the authentic sample, Compound 7 was obviously conclude to be β-amyrin. The structure of Compound 7 was shown below.



Compound 7 ß-Amyrin

---

* The authentic β-amyrinacetate was isolated and identified from the leaves of Streblus asper Lour. by Assist. Prof. Gaysorn Veerachato.

3.3.8 Structural Elucidation of Compound 8

Compound 8, 2.64 g. (1.76% wt. by wt. of Fraction I) was isolated from 10% chloroform-hexane by repeat column chromatography of Fraction F and purified by recrystallization from hot n-hexane to gain white feather product with wide melting range 192.0-210.0 °C. To aid the purification, the acetyl derivative was synthesized to yield Compund 8 acetate, m.p. 216.0-218.0 °C, Rf 0.69 (solvent: 50% chloroform-hexane). After hydrolysis Compound 8 acetate by using 10% KOH in ethanol, Compound 8 was derived as bright white needle crystal, m.p. 214.0-215.0 °C, Rf 0.37 using chloroform as a developing solvent. Compound 8 gave a violet colour with Liebermann-Burchard's and also decolourized Br_2 in CCl_4 reagents. This result hinted the presence of an unsaturated triterpenoid (55,60-61).

The IR spectrum (Fig.68) which is assigned in Table 3.15 revealed the characteristic absorption bands of 2 OH group (probably equatorial) (66-67) at 3400-3200 and 1035 cm^{-1} and additional bands of a vinylidine group (CH_3-C=CH_2) at 3090, 1640 and 875 cm^{-1} (92) together with gem dimethyl group at 1380 and 1180 cm^{-1}.

The UV spectrum (Fig.71) gave the λ max at 205 nm. (log ε = 3.64), this data showed the presence of non-conjugated system in this compound (70).

Table 3.15 The IR absorption band assignments of Compound 8

Frequency (cm^{-1})	Band type	Tentative assignments
3400-3200	very b.	O-H stretching vibration
3090	w	C-H stretching vibration of asymmetric $R_1R_2C=CH_2$
2970-2870	s	C-H stretching vibration of CH_3, $-CH_2-$
1640	m	non-conjucated C=C stretching vibration
1480-1430	s	C-H bending vibration of CH_3, $-CH_2-$
1380	s	C-H bending vibration of gem-dimethyl
1180	m	skeleton vibration of $(CH_3)_2-C<$ with no free hydrogen atom on the central carbon
1035	s	C-O stretching vibration of 3 β -OH (equatorial)
875	s	C-H out of plane bending vibration of $=CH_2$

The ^1H NMR spectrum (Fig.69) gave good agreements with the IR spectrum information, i.e., it exhibited the singlet signals of six methyl protons at δ 0.76, 0.79, 0.83, 0.94, 0.96 and 1.03 ppm. (3H each), the broaden singlet of vinylic methyl proton at 1.68 (3H) (92), the broad multiplet of a methine proton attached to a carbon atom bearing a hydroxyl group (-CH-OH) at δ 3.16 ppm. (1H) and the two signals in olefinic region which should be a terminal

methylene proton (CH_2=C-) at 4.67 and 4.57 ppm. (2H,J=9.00 Hz). The ^1H NMR chemical shift assignments of compound 8 and Compound 8 acetate are given in Table 3.16.

Table 3.16 The ^1H NMR chemical shift assignments of Compound 8 and Compound 8 acetate

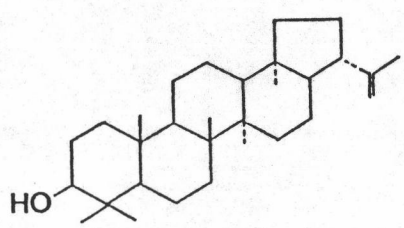
Compound	Chemical shifts (ppm.)			
	olefinic protons	carbinol proton	vinylic methyl proton	methyl protons
lupeol	4.68,4.78 (2H,J=7.00 Hz)	3.13	1.70	0.78(3H),0.80(3H) 0.84(3H),0.98(6H) 1.05(3H)
Compound 8	4.67,4.57 (2H,J=9.00 Hz)	3.16(1H, t,J=7.82 Hz)	1.68 (3H,s)	0.76,0.79,0.83, 0.94,0.96,1.03 (3H each)
Compound 8 acetate	4.67,4.55 (2H,J= 10.26 Hz)	4.42(1H, t,J=9.04 Hz)	1.68 (3H,s)	0.78, 0.94,1.03 (3H each), 0.84 (6H)

The mass spectrum (Fig.72) displayed the molecular ion (M^+) at m/e (% rel. int.) 426.0 (71.36) (Calcd. for $C_{30}H_{50}O$: MW. 426.39) together with other abundant fragmentation ions at m/e 383.0 (5.09), 207.0 (75.55), 189.0 (82.38) and 95.0 (100.00). The series of fragmentation pattern implied that this triterpenoid compound should belong to a lupane series, i.e., the fragmentation ion peak at m/e 383.0 was due to the lost of isopropenyl group (CH_3-C=CH_2) which was the most important characteristic of this type of triterpene compounds (73). The presence of the ion at m/e 207.0 indicated the hydroxy group ought to locate at C-3 (93). The

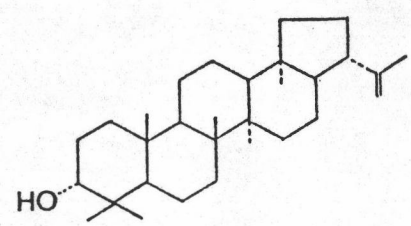
possible mass fragmentation pattern of Compound 8 was presented in Scheme 3.6.

The ^{13}C NMR spectrum (Fig.70) exhibited the olefinic carbon signals at 150.88 and 109.32 ppm.. In the ^{13}C NMR off resonance spectrum (Fig.70b), it still showed a siglet signal at the former position which suggested that there was no hydrogen attached on that carbon, whereas the latter gave a triplet signal which was indicative the presence of a structure like $=CH_2$. The singlet signal at 78.99 ppm. which was shown a doublet signal in the ^{13}C NMR off resonance suggested the structure like $\geq CH\text{-}OH$. Besides, there were other signals around 55.37 to 14.57 ppm. which were the signals of methyl, methylene, methine and quarternary carbons.

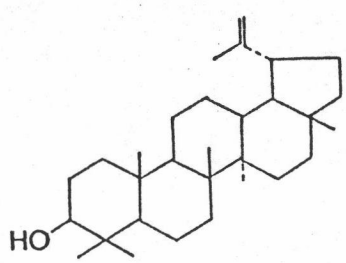
The information above was obviously proved that Compound 8 possed a lupane pentacyclic triterpenoidal skeleton with one equatorial hydroxy group at C-3, and one vinylidene moiety, probably in Ring E, together with six methyl groups. A literature search basing on these facts and its melting point narrowed the possibility of Compound 8 to one of the following compounds.



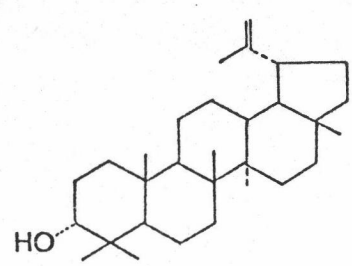
Moretenol



3-Epimorenol

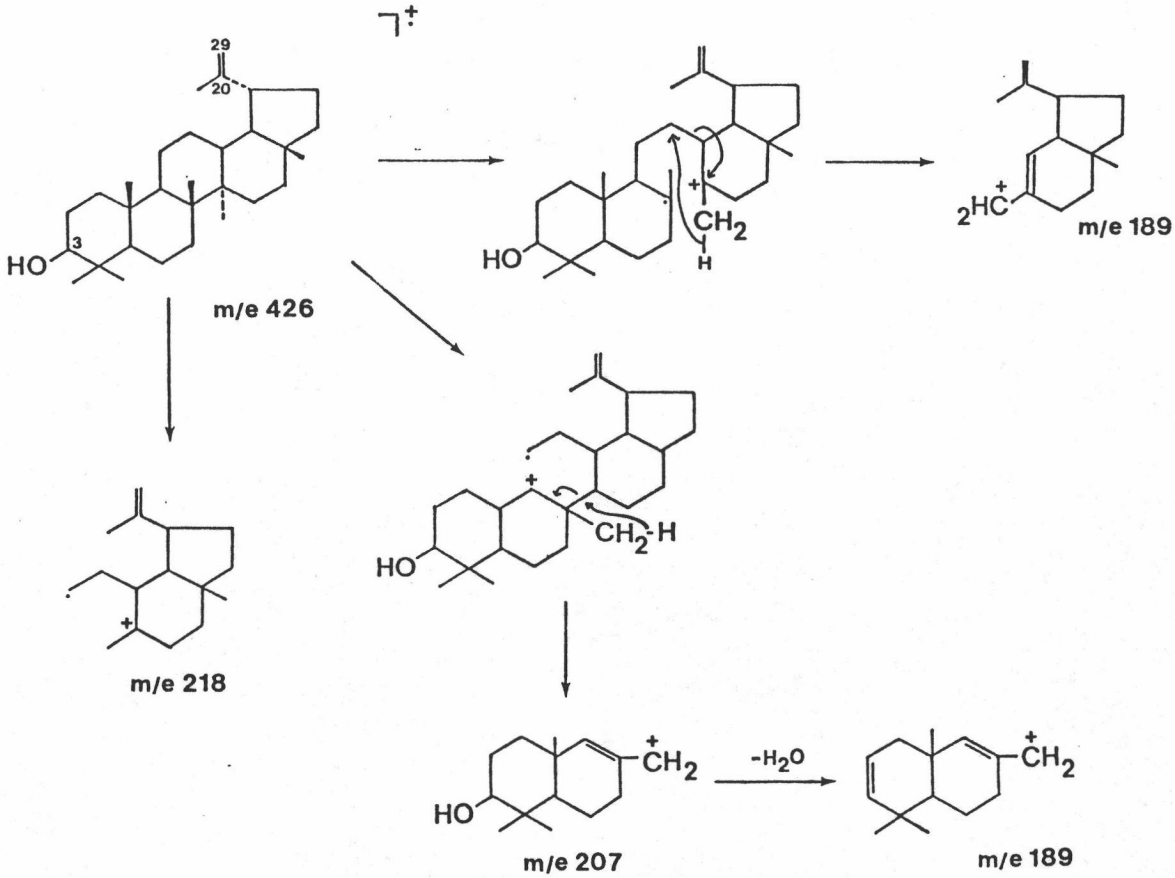


Lupeol



3-Epilupeol

Scheme 3.6 The possible mass fragmentation pattern of Compound 8



Since a lack of the authentic samples of these compounds except lupeol which was kindly supplied by Assist. Prof. Gaysorn Veerachato, the direct comparision, i.e., mixed m.p. and Co. TLC techniques was impossible to perform. Compound 8 was, hence, compared their melting point with these possible substances. The comparision data among moretenol, 3-epimoretenol, lupeol and 3-epilupeol is tabulated in Table 3.17.

Table 3.17 The comparision data among moretenol, 3-epimoretenol, lupeol, 3-epilupeol and Compound 8

Compound	melting point (°C)		Ref.
	alcohol	acetate	
moretenol	236	283-285	94,95
3-epimoretenol	223	233-234	95
lupeol	215-216	217-218	94
3-epilupeol	202	-	96
authentic lupeol*	212.0-213.5	216.0-217.5	-
Compound 8	214.0-215.0	216.0-218.0	-

From Table 3.17, it was found that Compound 8 was closely to be lupeol rather than other triterpenoids.

---

* The authentic lupeol and lupeolacetate was separated and characterized from the stembarks of Crataeva nurvala by Assist. Prof. Gaysorn Veerachato.

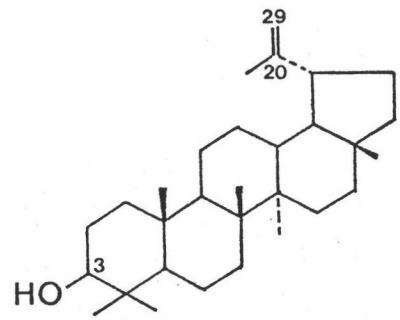
To confirm this observation, the mixed m.p., Co. TLC and the GLC analysis were carried out by comparing with the authentic ones. The results from these examinations were no doubt to state that Compound 8 and Compound 8 acetate were lupeol and lupeolacetate, respectively.

Moreover, the ^{13}C NMR chemical shifts of Compound 8 and Compound 8 acetate were corresponded to that of reported lupeol (97,98). The comparision of the ^{13}C NMR chemical shifts of lupeol, Compound 8 and Compound 8 acetate is presented in Table 3.18.

Table 3.18 The ^{13}C NMR chemical shift assignments of lupeol, Compound 8 and Compound 8 acetate

Carbon	Chemical shifts (ppm.)		
	lupeol	Compound 8	Compound 8 acetate
1	38.7	38.79	38.03
2	27.4	27.47	27.47
3	78.8	78.99	80.94
4	38.8	38.90	38.41
5	55.2	55.37	55.37
6	18.3	18.36	18.20
7	34.2	34.35	34.24
8	40.8	40.90	40.85
9	50.4	50.49	50.33
10	37.1	37.22	37.11
11	20.9	21.02	20.97
12	25.1	25.19	25.08
13	38.0	38.14	38.03
14	42.8	42.85	42.85
15	27.4	27.47	27.47
16	35.5	35.65	35.59
17	42.9	43.01	42.85
18	48.2	48.38	48.27
19	47.9	48.00	48.00
20	150.6	150.88	150.87
21	29.8	29.90	29.85
22	39.9	40.03	39.98
23	28.0	28.01	27.95
24	15.4	15.38	15.98
25	16.1	16.14	16.52
26	15.9	16.04	16.20
27	14.5	14.57	14.52
28	18.0	18.04	18.04
29	109.2	109.32	109.38
30	19.3	19.34	19.29
$-O_2\underline{C}-CH_3$	-	-	170.97
$\underline{CH_3}-CO_2\underline{-}$	-	-	21.29

The above spectral evidences together with colour tests and chemical reactions supported that Compound 8, one of the major compounds of Fraction I, had to be lupeol and its structure is drawn as follows:



Compound 8 Lupeol

3.3.9 Structural Elucidation of Compound 9

Compound 9, recrystallized by a mixture of n-hexane and ethylacetate to yield bright colourless prism crystal or by a mixture of chloroform and n-hexane to gain bright white needle crystal 5.67 g. (3.78% wt. by wt. of Fraction I), m.p. 278.0-280.0 °C, Rf 0.34 (solvent: chloroform), was obtained from the separation by silica gel recolumn chromatography of Fraction F. This compound gave a violet colour with Liebermann-Burchard's and also decolourized Br_2 in CCl_4 reagents which were indicative of a triterpenoidal skeleton as well as an unsaturation part in its structure (55,60-61).

The IR spectrum (Fig.74), assigned in Table 3.19, revealed the important characteristic bands belonging to 2 OH (may be equatorial) at 3490, 1040 and 1005 cm^{-1} (87,99) and additional bands attributed to trisubstituted olefinic moiety at 3050, 1645 and 815 cm^{-1} (66,67).

Table 3.19 The IR absorption band assignments of Compound 9

Frequency (cm^{-1})	Band type	Tentative assignments
3490	m	O-H stretching vibration
3050	w	C-H stretching vibration of $R_1R_2C=CR_3H$
2995,2940	s	C-H stretching vibration of CH_3-
2870,2850	s	C-H stretching vibration of $-CH_2-$
1645	w	C=C stretching vibration
1475,1385	s	C-H bending vibration of CH_3- and $-CH_2-$
1040,1005	s	C-O stretching vibration of 3 β-OH
815	w	C-H out of plane bending of trisub. olefin

The ^1H NMR spectrum (Fig.77) displayed the important signals at 5.53 ppm. (1H,dd.,J=3.43,7.55 Hz) attributed to the signal of an olefinic proton and at 3.21 ppm. (1H,t,J=6.93 Hz) compatible with the signal of a proton on carbon which attached to oxygen atom. The eight methyl proton signals were observed at 0.81, 0.83, 0.91, 0.93, 0.95, 0.98 (3H each) and 1.10 ppm. (6H). The tentative assignments of the ^1H NMR chemical shifts are given in Table 3.20 (100).

The UV spectrum (Fig.75) gave the λ max at 204 nm. (log ε = 3.72) which suggested the presence of non-conjugated unsaturation system (70).

Table 3.20 The ^{1}H NMR chemical shift assignments of Compound 9, Compound 11 and Compound 12

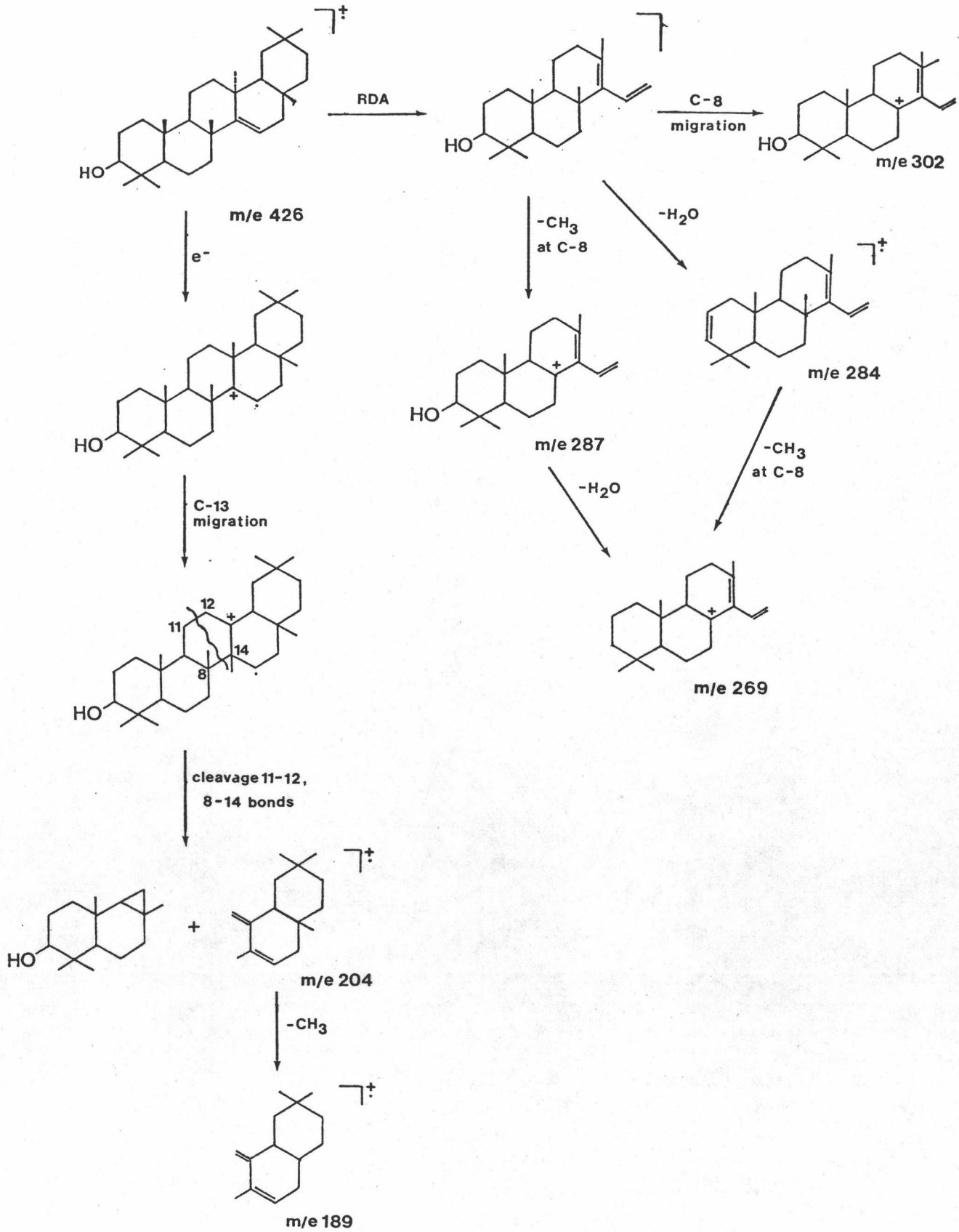
Compound	Chemical shift (ppm.)						Remarks
	olefinic proton	C-3 proton	methyl protons	p-coumaryl moiety			
				aromatic protons	olefinic protons	phenolic proton	
taraxerol	5.54 (1H)	3.22 (1H)	0.81,0.83,0.91,0 0.93,0.95,0.98 (3H each),1.10 (6H)	-	-	-	Ref. 100
Compound 9	5.53 (1H,dd J=3.43,7.55 Hz)	3.21 (1H,t, J=6.93 Hz)	0.81,0.83,0.91, 0.93,0.95,0.98 (3H each),1.10 (6H)	-	-	-	Fig. 77
Compound 9-OAc	5.52 (1H,dd J=3.42,7.96 Hz)	4.45 (1H,t, J=7.80 Hz)	0.82,0.86,0.90, 1.02,1.08,1.20 (3H each),0.95 (6H)	-	-	-	Fig. 80
careaborin	5.50 (1H,dd, J=4.00,8.00 Hz)	4.60 (1H,dd, J=5.00,9.00 Hz)	0.83,1.10 (3H each 0.90,0.95,0.97 (6H each)	6.89,7.33 (2H each, d, J=8.00 Hz)	6.33,7.60(1H each, d,J=16.00 Hz)	*	Ref. 119
Compound 11	5.50 (1H,dd, J=2.56,7.05 Hz)	4.52 (1H,t, J=5.88 Hz)	0.84,0.87,0.93, 1.09 (3H each), 0.91,0.95 (6H each)	5.82,6.83(2H each, d,J=11.69 Hz)	6.75,7.61(1H each, d,J=8.55 Hz)	5.42	Fig. 95
Compound 12	5.54 (1H,dd, J=3.22,8.87 Hz)	4.59 (1H,t, J=7.39 Hz)	0.82,0.91,0.93, 1.10 (3H each),0.91, 0.96 (6H each)	6.84,7.43(2H each, d,J=8.30 Hz)	6.29,7.61(1H each, d,J=16.12 Hz)	5.48	Fig. 99

* The chemical shift of the phenolic proton of careaborin did not report

The molecular formular of this compound was proposed to be $C_{30}H_{50}O$ (MW. 426.39). This formular gave good agreement by both the mass spectrum data as well as the elemental analysis result.

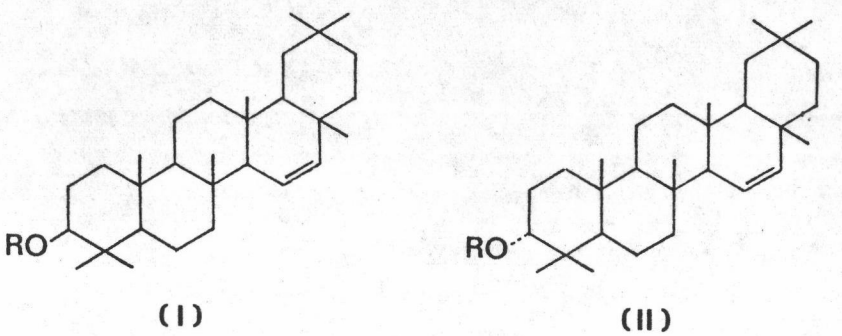
The mass spectrum of this compound (Fig.76) exhibited the parent ion peak at m/e (% rel.int.) 426.0 (24.02) (Calcd. for $C_{30}H_{50}O$: MW. 426.39) and other fragmentation ion peaks at m/e 411.0 (10.90, M^+-CH_3), 408.0 (1.96, M^+-H_2O), 393.0 (3.20, $M^+-CH_3-H_2O$), 302.0 (45.68, RDA), 287.0 (30.70, RDA-CH_3), 269.0 (13.87, RDA-CH_3-H_2O), 204.0 (100.00, cleavage of 11-12 and 8-14 bonds) and 189.0 (21.41, 204-CH_3). The series of fragmentation ion pattern at m/e 302.0, 269.0, 204.0 and 189.0 implied that this triterpenoid should be a member of taraxerane triterpenoidal compounds (73). The possible mass fragmentation pattern of Compound 9 is presented in Scheme 3.7.

Scheme 3.7 The possible mass fragmentation pattern of Compound 9



The ^{13}C NMR spectrum (Fig.78) displayed the olefinic carbon signals positioned at δ 158.00 and 116.77 ppm. From the ^{13}C NMR off resonance spectrum, the signal at 158.00 ppm. still showed only one peak, whereas the signal at 116.77 ppm. exhibited a doublet peak due to the coupling with one proton. This information revealed that there was a structure like $>C=C<^{H}$ in this molecule. The signal at 78.96 ppm. was corresponded to the carbon adjacent to hydroxy group. The rest signals could be assigned for methyl, methylene, methine and quartenary carbons (72). The ^{13}C NMR chemical shift assignments of this compound are presented in Table 3.21 (101,102).

Literature searches through triterpenoids distributed in mangrove plants, especially in Rhizophoraceae family as cited in Table 1.2 and Fig. 4, found that the corresponded triterpenoid basing on its structural formular and a basic skeleton of taraxerane group was taraxerol (I) (32). However, other chemical literatures reported that the isotaraxerol (II) was also occurred in natural sources (103). These two compounds were different at the configuration of -OH at C-3.



(I) (II)

(I),(II) R = H

Table 3.21 The ^{13}C NMR chemical shift assignments of Compound 9

Carbon	Chemical shifts (ppm.)	
	taraxerol	Compound 9
1	38.7	38.65
2	27.3	27.01
3	79.1	78.96
4	38.8	38.65
5	55.6	55.45
6	18.9	18.66
7	33.2	33.02
8	39.1	38.87
9	49.4	49.16
10	37.6	37.46
11	17.6	17.42
12	36.8	36.54
13	37.8	37.90
14	158.2	158.00
15	117.0	116.77
16	33.8	33.50
17	35.8	35.68
18	48.9	48.68
19	41.4	41.25
20	28.8	28.68
21	35.2	35.02
22	37.8	37.90
23	28.0	27.93
24	15.5	15.36
25	15.5	15.20
26	26.0	25.87
27	29.7	29.82
28	29.9	29.82
29	33.4	33.42
30	21.4	21.26

The best information that could be distinguished these two triterpenes was the signal belonging to the carbinol proton and its coupling constant in the ^1H NMR spectrum (88). Hence, the signal at 3.21 ppm. (t,1H,J=6.93 Hz) in the ^1H NMR of Compound 9 was the important signal to show this expected difference. In isotaraxerol (II), the carbinol proton appeared at 3.38 ppm. with J = 1-5 Hz., typical of an equatorial proton associated with 3 α-hydroxy group in Ring A of a triterpenoid, whereas the C-3 proton in taraxerol (I) appeared at δ 3.22 ppm. with J = 8-13 Hz. corresponded to an axial proton associated with a 3 β-hydroxy group (100). This data was lucidly to point out that Compound 9 had to be taraxerol (I).

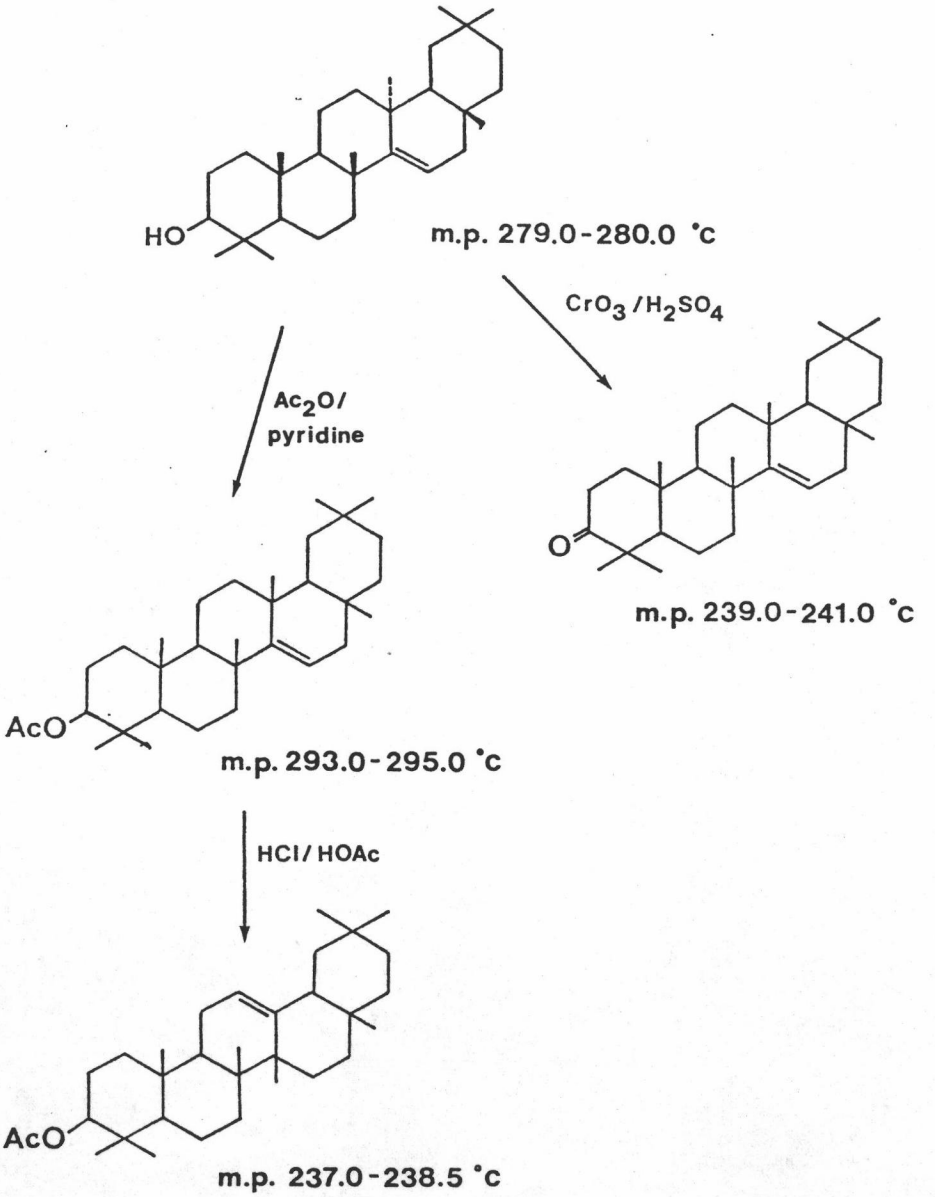
To confirm the major constituent of this fraction, a set of the derivatives of Compound 9, taraxerone and taraxerylacetate (I, R = O and OAc, respectively) were prepared and compared with those reported ones. The results found that the synthetic ones were acceptable to be expected compounds.

Another significant characteristic of taraxerol is its rearrangement to β-amyrin with HCl-HOAc (104). This reaction was also supported that if Compound 9 was taraxerol, it should derive β-amyrin from this reaction, while if it was isotaraxerol, epi-β-amyrin ought to be obtained The result from the reaction by using taraxerylacetate as a substrate found that the final product was identical to the authentic β-amyrinacetate*. All chemical reactions of Compound 9 are shown in Scheme 3.8.

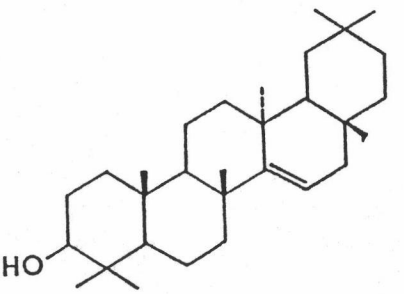
---

* Identified by mixed m.p., Co. TLC and the GLC analysis with the authentic one.

Scheme 3.8 Chemical reactions of Compound 9



According to physic chemical properties of this compound, this major constituent of Fraction I had to be taraxerol. The structure of this triterpenoid is shown as follow



Compound 9 Taraxerol

3.3.10 Structural Elucidation of Compound 10

Compound 10 was collected from the Fraction G which was eluted by the silica gel column chromatography of Fraction ID and recrystallized from a mixture of dichloromethane and n-hexane to gain bright white needle crystal, m.p. 143.0-145.0 °C, 550 mg. (0.36% wt. by wt. of Fraction I). It revealed merely one spot at Rf 0.32 using chloroform as a mobile phase. This compound gave a deep green colour with Liebermann-Burchard's and also decolourized Br_2 in CCl_4 reagents. This result suggested the possibility of unsaturated steroid (55,60-61).

The IR spectrum (Fig.85) gave the characteristic absorption bands of 2° ROH (probably equatorial) (95,105) at 3430, 1050 and 1020 cm^{-1}, and additional bands at 1650 cm^{-1} might due to the C=C stretching vibration of a trisubstituted olefin structure (67,81). The significant absorption bands at 840 and 800 cm^{-1}

(probably C-H out of plane bending), coincided with the characteristic absorption peaks of Δ^5-3β-hydroxy steroids (106, 107). The IR absorption band assignments of this compound is presented in Table 3.22.

The UV spectrum (Fig.89) gave the max at 203 nm. (log = 3.78) which indicated the presence of non-conjugated olefin in this compound (70).

Table 3.22 The IR absorption band assignments of Compound 10

Frequency (cm^{-1})	Band type	Tentative assignments
3430	b	O-H stretching vibration
3020	very w	C-H stretching vibration of $R_1R_2C=CR_3H$
2960-2840	s	C-H stretching vibration of $-CH_3$, $-CH_2-$
1650	w	C=C stretching vibration
1460,1370	m	C-H bending vibration of $-CH_3$, $-CH_2-$
1050	m	C-O stretching vibration of 3 β-OH equatorial position
970,960	m	C-H out of plane bending vibration of trans configuration
840,800	w	C-H out of plane bending vibration

Both the colour tests and the IR spectral information seem to support a steroidal structure. Moreover, the IR spectrum was found to be close to that of β-sitosterol (78). On thin layer chromatography, Compound 10 gave the same Rf value as the

authentic β-sitosterol at 0.32 and 0.28 using chloroform and a mixture of n-hexane and diethylether (3:2) as developing solvents. This strongly indicated that Compound 10 might be β-sitosterol. It was not surprising to isolate this substance, since this steroid is widely distributed in the plant kingdom (108) and S.G. Majumdra and G. Patra (32) had reported that this steroid was present in the leaves of this particular species.

Nevertheless, the m.p. of Compound 10 (143.0-145.0 °C) differed from the authentic sample (m.p. 134.0-136.0 °C) (Lit (74) 134.0 °C) and some peaks on the IR spectrum were different. In the IR spectrum of Compound 10, the doublet peaks were observed at 970 and 960 cm^{-1}, while in that of the authentic one gave the singlet peak at 970 cm^{-1} (109). This result was notified and suggested that Compound 10 ought to be composed of at least two different steroid compounds.

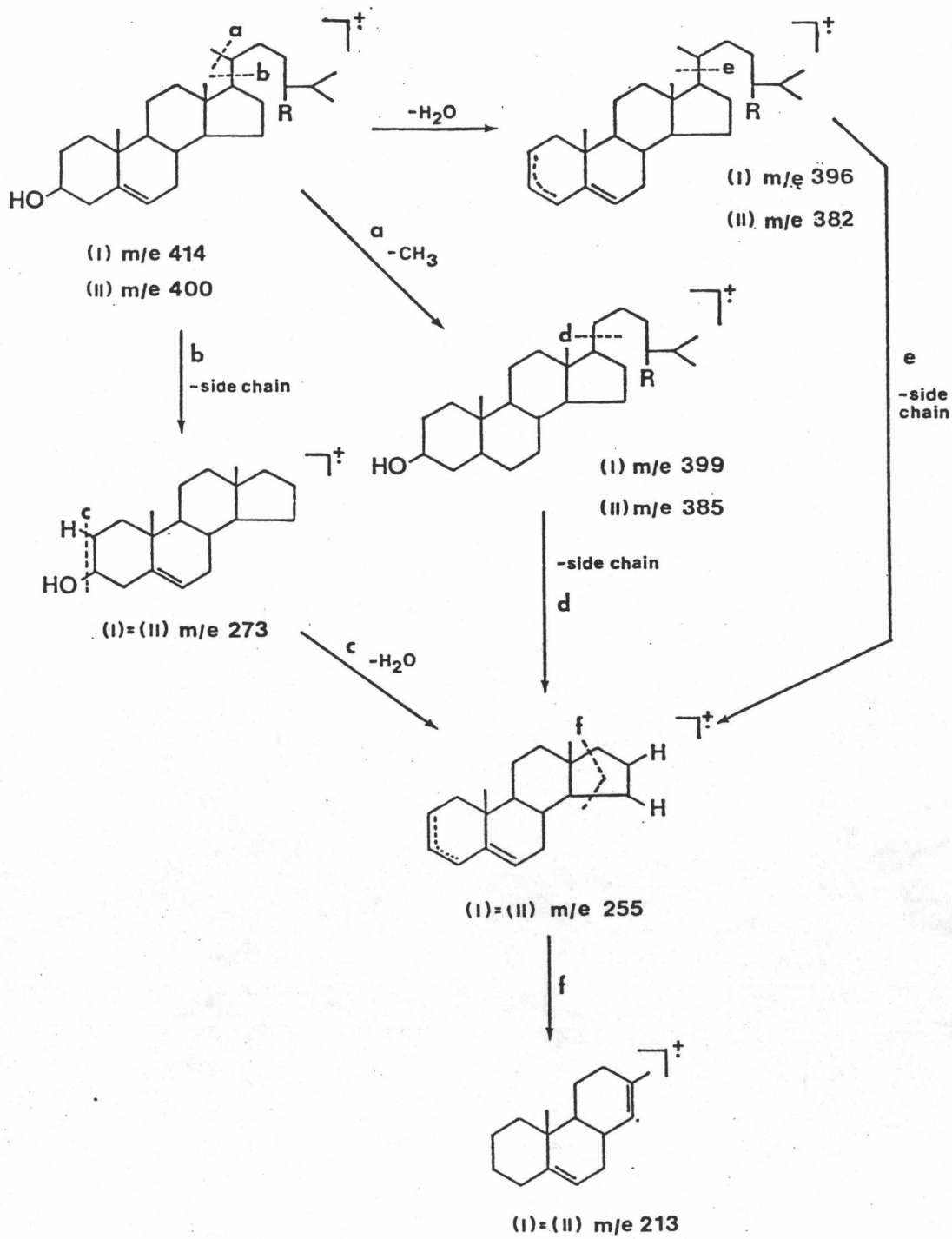
The ^{1}H NMR spectrum of Compound 10 (Fig.87) was coincided with that of β-sitosterol except the signal at 5.09 ppm. which might be due to other olefinic protons. Other signals could be assigned as follows:the signal at 5.32 ppm. was thought to be the signal of vinylic proton attached to a methylene group at C-6 in β - sitosterol ($>C=CH-CH_2-$). A broad multiplet at δ 3.52 ppm. with an integration for 1H was typical for a hydrogen geminal to a hydroxy group at C-3 ($>CH-OH$); a singlet at 1.57 ppm. disappeared upon shaking with D_2O indicating the presence of hydroxylic proton at C-3. Other signals around 2.29 to 0.68 ppm. corresponded to the proton signals of methyl, methylene and methine protons (110).

The information from the ^{1}H NMR spectrum supported the IR spectrum result that Compound 10 should be consisted of more than one steroid.

The mass spectrum (Fig.86) displayed the expected molecular ion peak for β-sitosterol at m/e 414.0 (100.00) and also revealed the fragmentation pattern of C_{29} steroids (111-112). However, two significant peaks which could not be accounted for the β-sitosterol structure was observed at m/e 412.0 (80.23) and m/e 400.0 (40.83). Since the loss of two atoms of hydrogen from the molecular ion is highly unlike, there must be a second component corresponding to the fragmentation ion peak at m/e 412.0. Similarly, the loss of a methylene fragment from the ion m/e 414.0 and a unit of 12 from the ion m/e 412.0 could be ruled out, therefore, the ion at m/e 400.0 indicated the presence of a third compound. The possible mass fragmentation pattern of Compound 10 is shown in Scheme 3.9.

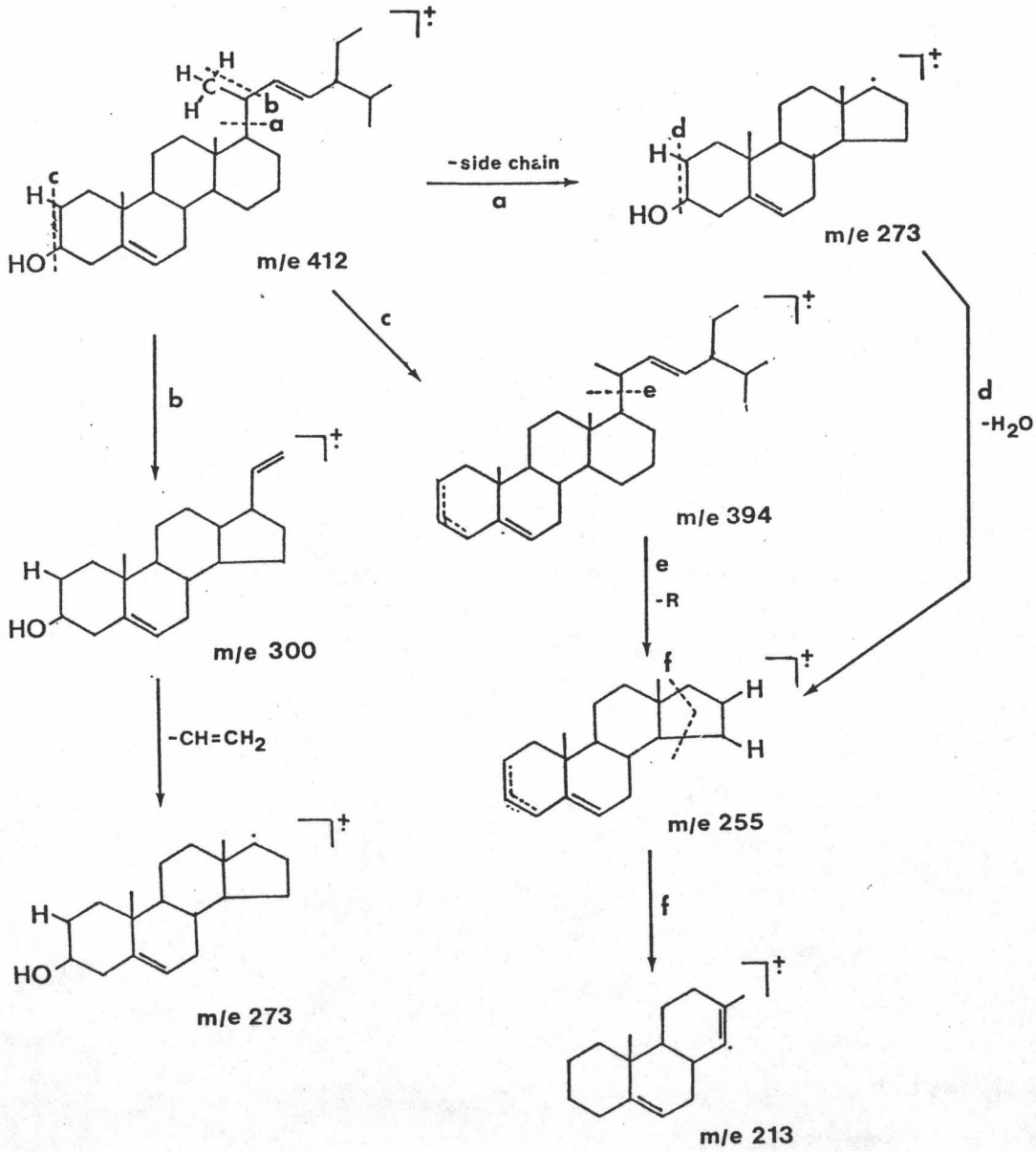
Scheme 3.9 The possible mass fragmentation pattern of Compound 10

for β-Sitosterol (R = C_2H_5) and Campesterol (R = CH_3)



Scheme 3.9 (Cont.)

for stigmasterol



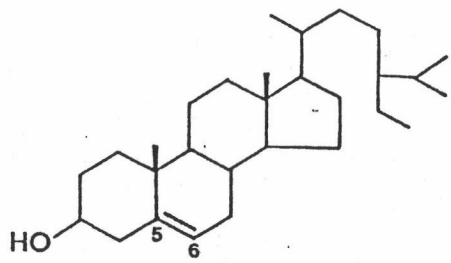
Thin layer chromatography employing various developing solvent systems failed to separate this mixture.

A search through the literatures revealed that the presence of β-sitosterol in plants was frequently accompanied by one or more than steroids (108,113-114) such as stigmasterol and campesterol. In order to prove the presence of mixed steroids, two ways of analysis could be used. The first one was using the high resolution mass spectrometer which could measure the accurate mass for the molecular ion and calculated the possible molecular formular from their isotope abundants (68). The other way which was selected to prove the structure of Compound 10 was using gas-liquid chromatography. From the GLC chromatogram of Compound 10 three major peaks were exhibited at retention time (Rt) 18.40, 19.60 and 22.30 min., respectively which were corresponded to the authentic campesterol, stigmasterol and β-sitosterol*, respectively.

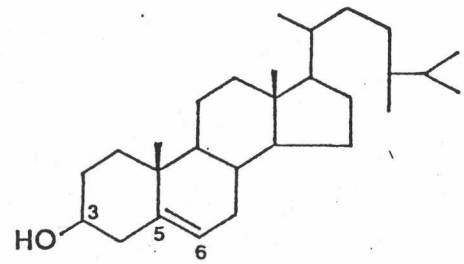
Hence, it was obvious to conclude that Compound 10 was a mixture of β-sitosterol, stigmasterol and campesterol. The structure of these three steroids are given as follows:

---

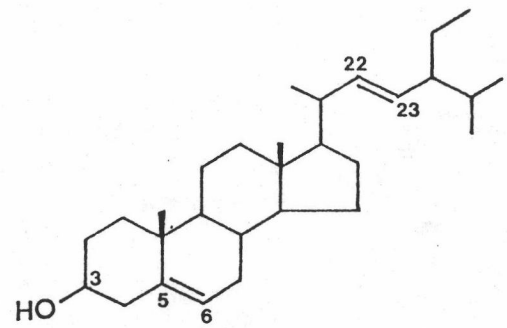
* The authentic stigmasterol, campesterol and a mixture of β-sitosterol and campesterol are derived from Sigma Chemical Company.



ß-Sitosterol



Campesterol



Stigmasterol

Compound 10

The composition of steroids in Compound 10 is presented in Table 3.23.

Table 3.23 The composition of steroids in Compound 10

Name	Rt (min.)	% Composition
campesterol	18.40	6.96
stigmasterol	19.60	38.45
ß-sitosterol	22.30	54.60

A literature study reported that the ^{13}C NMR spectrum of stigmasterol showed 24 carbons, while that of β-sitosterol gave 27 carbons. The ^{13}C NMR chemical shifts of campesterol were superimposed to that of β-sitosterol. However, the spectrum of a mixture of stigmasterol and β-sitosterol gave 37 carbon signals because of the different environments of some methyl, methylene, methine and quarternary carbons around 11.86-56.86 ppm. The olefinic carbons of β-sitosterol was on those of stigmasterol as well as the carbinol signal. The ^{13}C NMR chemical shifts of Compound 10 could be assigned as presented in Table 3.24 (109,115).

Table 3.24 The ^{13}C NMR chemical shift assignments of Compound 10

Carbon	Chemical shifts (ppm.)			
	stigmasterol	β-sitosterol	Compound 10	
			stigmasterol	β-sitosterol*
1	37.38	37.25	37.27	37.27
2	31.75	31.66	31.69	31.69
3	71.84	71.77	71.78	71.78
4	42.32	45.84	42.31	45.88
5	140.81	140.72	140.80	140.80
6	121.74	121.64	121.72	121.72
7	31.97	32.89	31.91	31.91
8	31.97	32.89	31.91	31.91
9	50.27	50.14	50.16	50.16
10	36.57	36.51	36.51	36.51
11	21.13	19.39	21.13	19.39
12	39.77	39.82	29.16	29.20
13	42.32	45.84	42.31	45.88
14	56.94	56.75	56.77	56.77
15	24.40	24.29	24.32	24.32
16	28.93	39.76	28.93	39.82
17	56.08	56.07	56.07	56.07
18	12.24	11.97	12.24	12.03
19	19.45	19.04	19.39	19.07
20	40.53	33.95	40.52	33.97
21	21.13	18.78	21.13	18.80
22	138.32	36.15	138.31	36.19
23	129.38	23.07	129.31	23.08
24	51.31	42.31	51.25	42.31
25	31.97	28.24	31.91	28.28
26	19.02	21.10	19.07	21.13
27	21.13	19.80	21.13	19.83
28	25.47	26.11	25.41	26.11
29	12.08	11.86	12.03	11.86

* The ^{13}C NMR signals of campesterol were superimposed on those of β-sitosterol.

3.3.11 Structural Elucidation of Compound 11

Compound 11 was isolated from silica gel column chromatography of Fraction H1, recolumn chromatography of Fraction H2 and the upper part obtained from the PTLC plate. After recrystallization with a mixture of chloroform and n-hexane for several times, Compound 11 as white amorphous solid, 50 mg. (0.03% wt. by wt. of Fraction I), m.p. 275 °C (dec.), Rf 0.19 (solvent: chloroform), was obtained. This compound gave a violet colour with Liebermann-Burchard's, a deep green colour to 5% $FeCl_3$ and also decolourized Br_2 in CCl_4 reagents. The colour tests suggested that this compound should be composed of triterpenoidal skeleton, phenolic group and unsaturation part (55,60-61).

The IR spectrum (Fig.92), assigned as Table 3.25, revealed the important characteristic peaks which were corresponded to an ester compound (1715 (-$\overset{O}{\overset{\|}{C}}$-O-), 1200 and 1045 (C-O)), phenolic moiety (3600 (O-H), 3050 (C-H of aromatic), 1630, 1605, 1520 (C=C), 1190, 1170 (C-O of phenolic compounds)). The absorption bands belonging to gem-dimethyl were observed at 1390 and 1380 cm^{-1}. The absorption peaks at 845 and 820 cm^{-1} pointed out C-H out-of-plane deformation of benzene ring (p-substitution, 2H adjacent) (66,67).

Table 3.25 The IR absorption band assignments of Compound 11

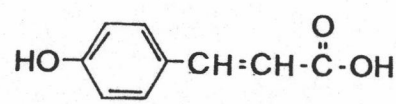
Frequency (cm^{-1})	Band type	Tentative assignments
3600-3570	s	O-H stretching vibration
3050	w	C-H stretching vibration of alkene or aromatic
2950,2865	s	C-H stretching vibration of CH_3-, -CH_2-
1715	s	-$\overset{O}{\overset{\|}{C}}$-O- stretching vibration of ester
1630,1605 1520	s	C=C stretching vibration of alkene or aromatic
1445,1340	m	C-H bending vibration of CH_3-, -CH_2-
1390,1380	m	C-H bending vibration of gem-dimethyl group
1270-1250	m	C-O stretching vibration
1190,1170	s	C-O bending vibration of phenolic gr.
845,820	m	C-H out of plane bending vibration of benzene ring (p-substitution)

From the colour tests and the IR spectrum suggested that this compound ought to be an ester of triterpene adjacent to phenolic carboxylic acid.

The mass spectrum (Fig.93) showed the molecular ion peak at m/e (% rel. int.) 572.0 (13.02) in conformity with its proposed molecular formular $C_{39}H_{56}O_3$ (MW. 572.44), together with other fragmentation ion peaks at m/e 448.0 (13.50), 408.0 (20.33,

$M^+-C_9H_8O_2$), 284.0 (31.34), 269.0 (33.36), 204.0 (94.90), 164.0 (30.86, M^+-408) and 147.0 (100.00). From the mass spectrum, the important fragmentation pattern at m/e 408.0, 204.0 and 189.0 strongly indicated the possibility of taraxerol as the triterpenoid of this compound (see also Compound 9). Since the most significant fragmentation of ester substances always obtain from the α- and β-cleavage (63,70), the acidic part of this compound should have a molecular weight equal to 164.0 (M^+-408) which was corresponded to $C_9H_8O_3$*.

From the colour tests revealed that there should be a phenolic group in this compound and the IR spectrum stated that this position of substituents on benzene nucleus should be p-substitution. The appropriate structure for the phenolic carboxylic acid ($C_9H_8O_3$), thus, should be p-coumaric acid. (p-hydroxycinnamic acid, I)



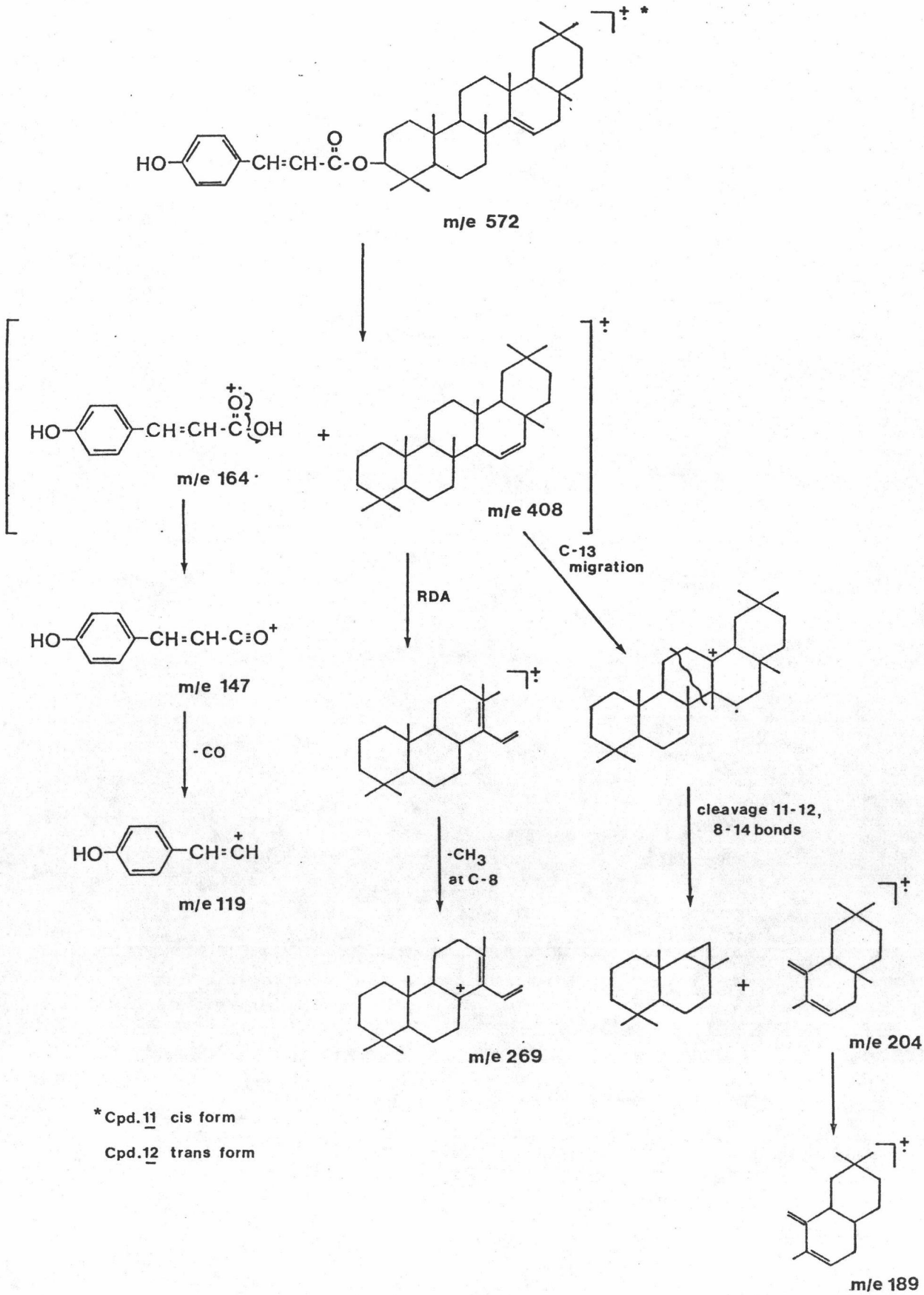
(I)

The presence of p-coumaric acid was supported by both the mass and the UV spectra. In the mass spectrum, the series of fragmentation ion peaks which were corresponded to p-coumaric acid were observed at m/e 164.0 (30.86), 147.0 (100.00) and 119.0 (29.31) (70,116). The possible mass fragmentation of Compound 11 is presented in Scheme 3.10.

---

* The molecular formular of this acid was obtained from the substraction $C_{30}H_{48}$ (the alcoholic part, taraxerol) from the molecular formular of this compound. (presented in Scheme 3.10.)

Scheme 3.10 The possible mass fragmentation pattern of Compound 11 and 12

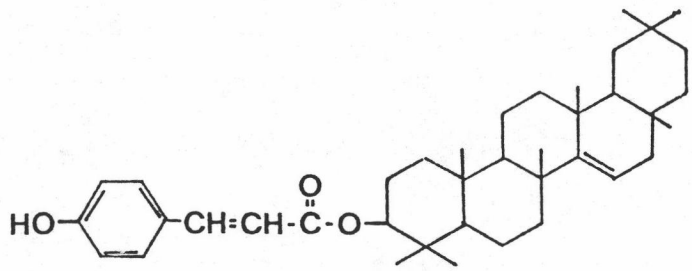


*Cpd. 11 cis form

Cpd. 12 trans form

The UV spectrum of this substance (Fig.94) gave the λ max at 312 nm. (log ε = 5.64), 208 nm. (log ε = 6.89) and a shoulder at 225 nm. (log ε = 4.14), the highest wavelength was shifted to 368 nm. (log ε = 4.11) in alkali medium (0.1 M. NaOH 3 drops). This result was clearly reminiscent of p-hydroxycinnamoyl moiety (108,117).

The ^{1}H NMR spectrum (Fig.95) gave good agreement with the structure of taraxeryl p-hydroxycinnamate (II), i.e., it



(II)

displayed a pair of 1H doublet signal centered at 7.61 and 6.75 ppm. (J=8.55 Hz) characterized of the olefinic protons of p-coumaryl moiety, the more downfield doublet was assignable to the proton at the β-position to carbonyl group. The next two signals at 6.83 and 5.82 ppm. (2H each,d, J=11.69 Hz) were attributable to the two meta hydrogens and two ortho ones, respectively. A double doublet signal centered at 5.50 ppm. (1H,J=2.56,7.05 Hz) was corresponded to the vinylic proton (-C=CH-CH_2-) in taraxerol (see also Compound 9). A singlet peak at m/e 5.42 ppm., disappeared upon shaking with D_2O, was fixed for a phenolic proton (71). The triplet signal at 4.52 ppm. (1H) was belonged to α-proton attached to oxygen atom in ester

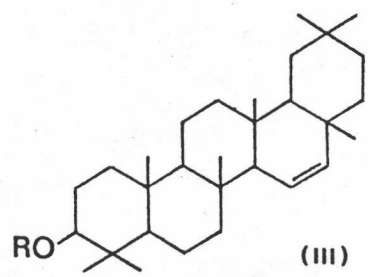
linkage ($R-\overset{O}{\overset{\|}{C}}-O-\underset{H}{\overset{|}{C}}-CH_2-$). The presence of eight methyl proton signals were observed at δ 0.84, 0.87, 0.93, 1.09 (3H each), 0.91 and 0.95 (6H each). The ^1H NMR chemical shift tentative assignments are presented in Table 3.20. Moreover, the coupling constant (J) belonging to the signal of olefinic protons in p-coumaryl moiety could be used to determine the geometry of this compound, i.e., the trans isomers always reveal the coupling constant (J) in range of 12-18 Hz, while the cis-form is lower in range of 6-12 Hz (71). At that position of Compound 11, the coupling constant equaled to 8.55 Hz which was indicated that the p-coumaryl moiety occurred in cis geometry to taraxerol.

The ^{13}C NMR spectrum (Fig.96) also agreed with other spectroscopic results, i.e, it revealed the carbon of ester at 166.53 ppm., the aromatic carbons and olefinic ones were present around 158.19 to 115.07 ppm.. Other carbon signals were coincided with the chemical shifts of taraxerol. The tentative assignments of the chemical shifts in the ^{13}C NMR spectrum are given in Table 3.26.

Table 3.26 The ^{13}C NMR chemical shift assignments of Compound 11 and Compound 12

Carbon	Chemical shift (ppm.)		
	taraxerol	Cpd. 11	Cpd. 12
1	38.7	37.69	37.49
2	27.3	23.56	23.62
3	79.1	81.21	81.04
4	38.8	38.08	37.76
5	55.6	55.85	55.74
6	18.9	18.80	18.74
7	33.2	33.26	33.15
8	39.1	39.11	39.06
9	49.4	49.35	49.24
10	37.6	37.60	37.60
11	17.6	17.61	17.55
12	36.8	36.84	36.73
13	37.8	37.87	37.98
14	158.2	158.19	157.59
15	117.0	117.02	116.96
16	33.8	33.86	33.75
17	35.8	35.86	35.81
18	48.9	49.08	48.86
19	41.4	41.44	41.39
20	28.8	28.88	28.82
21	35.2	35.27	35.19
22	37.8	37.87	37.98
23	28.0	28.12	28.06
24	15.5	16.63	16.79
25	15.5	15.55	15.55
26	26.0	25.95	25.95
27	29.7	29.96	29.90
28	29.9	29.96	29.90
29	33.4	33.42	33.37
30	21.4	21.34	21.34
1'	-	166.53	167.29
2'	-	115.07	115.88
3'	-	142.91	143.94
4'	-	118.10	116.96
5',9'	-	132.23	129.91
6',8'	-	115.07	115.88
7'	-	156.67	153.06

The search for the derivatives of taraxerol was carried out and found that almost isolated taraxerols were present in their alcohol (OH) (III, R=H) and acetate (III, R=OAc) forms or in its oxidative one -taraxerone (III, R = O), but seldom occurred in other forms. Thus, almost other types of taraxeryl derivatives which could be isolated from natural sources are usually novel derivative ones. To illustrate, R. Banerji et al. had separated a new lignoceric acid ester of taraxerol (IV) from the barks of Minujops littoralis (118). B. Talapatra and co-workers had isolated a new ester compound called "careaborin" (V) which was taraxeryl trans-p-hydroxycinnamate from the leaves of Careya arborea (119).

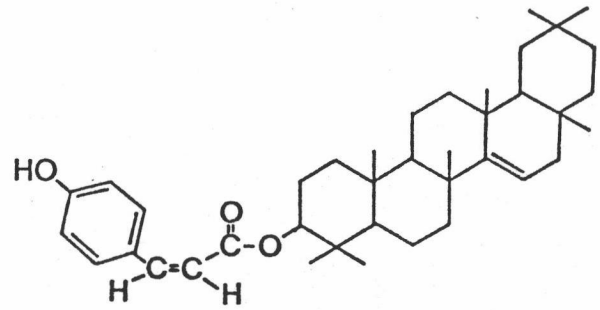


(III)

(IV) R= CH_3-$(CH_2)_{21}$-CH_2-CO-

(V) R= Trans-p-hydroxycinnamoyl

Although this compound was obtained in unsufficient amount to be confirmed by hydrolysis reaction, the spectroscopic data was lucid to establish the structure of Compound 11 to be taraxeryl cis-p-hydroxycinnamate. The structure of this compound is shown as follows:



Compound 11 Taraxeryl cis-p-hydroxycinnamate

To our knowledge, there is no report of taraxeryl cis-p-hydroxycinnamate in the chemical literatures. Hence, this substance is a novel naturally occurring ester of taraxerol.

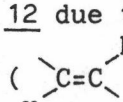
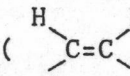
It was acquistioned that the cis form of almost substances were less stable compared with the trans ones, and sometimes not occurred. However, this puzzle was solved by using the CNDO calculation*. The result found that the cis form of this compound was stable enough to be existent, but less stable than the trans geometry (see Appendix I).

---

* The CNDO calculation was kindly made up by Dr. Sirirat Kokpol and Miss Supa Polman (Computational Chemistry Unit Cell, Department of Chemistry, Chulalongkorn University).

3.3.12 Structural Elucidation of Compound 12

Compound 12, m.p. 263 °C (dec.), was obtained from the combination of the lower part derived from PTLC and Fraction J. After purification by recrystallization with a mixture of chloroform and n-hexane for several times, white amorphous solid 210 mg. (0.14% wt. by wt. of Fraction I) was gained. This compound showed a single spot at Rf 0.14 using chloroform as a mobile phase. Like Compound 11, Compound 12 gave the same results to colour test reagents. This information indicated that Compound 12 should contain a triterpenoidal skeleton, phenolic group and unsaturation part (55,60-61).

The IR spectrum (Fig.97) which is assigned in Table 3.27, was similar to that of Compound 11. However, the important different absorption bands between these two compounds were observed, i.e., the strong absorption band at 970 cm^{-1} in Compound 12 due to C-H out-of-plane deformation of alkenes in trans geometry () was present, whereas this peak in the IR spectrum of Compound 11 (Fig.92) was absent. In compound 11, the C-H out-of-plane deformation of alkenes were detected at 845 cm^{-1} which could be accounted for the cis one ().

The molecular formular of Compound 12 was proposed as $C_{39}H_{56}O_3$ supported by the mass spectrum data and the elemental analysis result.

From these above data, the structure of this compound was firstly thought to be the trans isomer of Compound 11.

Table 3.27 The IR absorption band assignments of Compound 12

Frequency (cm^{-1})	Band type	Tentative assignments
3320	b	O-H stretching vibration
3050	w	C-H stretching vibration of alkene or aromatic
2940,2855	s	C-H stretching vibration of CH_3-, $-CH_2-$
1720	s	$-\overset{O}{\overset{\|}{C}}-O-$ stretching vibration of ester
1670,1640, 1615,1580, 1520	s	C=C stretching vibration of alkene and aromatic
1445,1340	s	C-H bending vibration of CH_3-, $-CH_2-$
1390,1380	m	C-H bending vibration of gem-dimethyl group
1270-1250	m	C-O stretching vibration of ester
1190,1170	s	C-O bending vibration of phenolic gr.
970	m	C-H out of plane bending vibration of alkene in trans geometry
820	s	C-H out of plane bending vibration of benzene ring (p-substitution)

The mass spectrum of Compound 12 (Fig.98) exhibited the same fragmentation pattern as that of Compound 11, i.e., it displayed M^+ at m/e (% rel.int.) 572.0 (8.36) together with other important peaks at m/e 408.0 (5.96, $M^+-C_9H_8O_3$), 204.0 (72.29) and 189.0 (24.65, $204-CH_3$). The series of fragmentation ion peaks, like

Compound 11, strongly pointed out the presence of taraxerol triterpenoid. The set of fragmentation ion peaks at m/e 164.0 (16.00, $C_9H_8O_3$), 147.0 (100.00) and 119.0 (27.49) revealed the existence of p-coumaric acid. The possible mass fragmentation pattern of Compound 12 is shown in Scheme 3.10.

The UV spectrum (Fig.101) also revealed the same result as Compound 11. It gave the λmax at 306 nm.(log ε = 4.39), 208 nm. (log ε = 4.19) and shoulder at 228 nm. (log ε = 4.09). The λ max at 306 nm. was shifted to 388 nm. (log ε = 4.19) in alkali medium (0.01 M. NaOH, 3 drops).

The information above agreed to each other that Compound 12 should be a trans isomeric structure of Compound 11.

To support this observation, the ^1H NMR spectrum (Fig.99) could be used as the best indicator to distinguish the difference of trans and cis isomer. Since the coupling constant belonging to trans isomer always gives in range 12-18 Hz, while the cis one does in range 6-12 Hz (71). As expected, the ^1H NMR of Compound 12 gave a pair of 1 H doublet signals centered at 7.61 and 6.29 ppm. (J=16.12 Hz) which was corresponded to trans olefinic protons of p-coumaryl moiety. The more down field doublet was attributed to the β-position to $-\overset{O}{\overset{\|}{C}}-O-$. Another pair of signal positioned at 7.43 and 6.84 ppm. (2H each,d,J=8.30 Hz) could be assigned for the two ortho and the two meta hydrogens, respectively. Other observed signals were similar to those of compound 11, i.e., a double doublet with 1H intensity at 5.54 ppm. was an olefinic proton, a singlet peak at 5.48 ppm. which disappeared when being shaken with D_2O belonged to the phenolic proton. A triplet peak

located at 4.59 ppm. was a proton bearing to oxygen atom in ester linkage. The eight methyl proton signals were present at 0.82, 0.91, 0.93, 1.10 (3H each), 0.91 and 0.96 (6H each). The tentative assignments of the ^{1}H NMR chemical shifts of this compound are shown in Table 3.20.

The ^{13}C NMR spectrum (Fig.100) gave the important carbon signals which could be assigned as follows: the ester carbon was located at 167.29 ppm., the signals around 157.59 to 115.88 ppm. could be fixed for the olefinic and the aromatic carbons, the carbon adjacent to oxygen of ester linkage was assigned at 81.04 ppm. together with other peaks around 55.74 to 15.55 ppm. which were identical to methyl, methylene, methine and quartenary carbons of taraxerol.

The structure of this compound was confirmed by basic hydrolysis with 10% KOH in ethanol, the alcoholic portion was undoubtedly found to be identical to taraxerol (Compound 9) by comparision of their IR, MS and conversion into its acetyl derivative. The acidic portion was proved clearly to be trans-p-coumaric acid by Co. TLC and the HPLC analysis compared with the authentic one*.

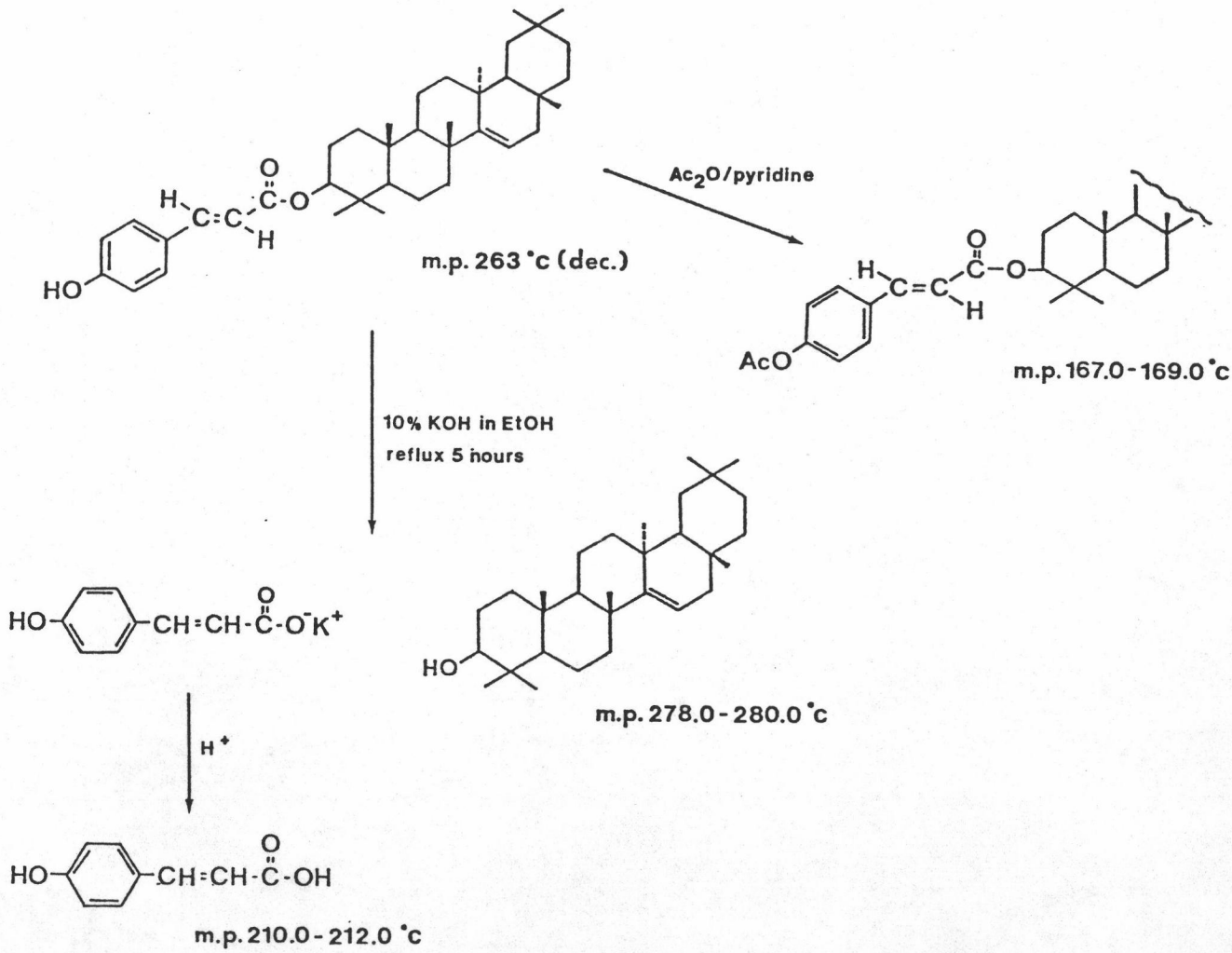
The acetyl derivative of Compound 12 was also prepared and found that the mass spectrum was lucidly supported the presence of a hydroxy group, i.e, the M^{+} was detected at m/e 614.0 (Calcd. for $C_{41}H_{58}O_{4}$: MW. 614.45). The other mass fragmentation

---

* The authentic p-coumaric acid is obtained from Fluka Chemie, Company.

pattern revealed the same pattern as Compound 12. All the chemical reactions of Compound 12 are written in Scheme 3.11.

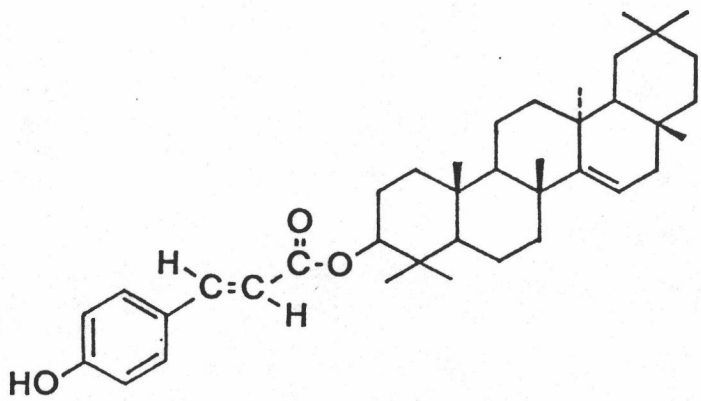
Scheme 3.11 The chemical reactions of Compound 12



As stated in Compound 11, careaborin or taraxeryl trans-p-hydroxycinnamate, was first isolated by B. Talapatra et al. from the leaves of Careya arborea in 1981 (119) and there was no report concerned with this substance furthermore. However, the ^{13}C NMR chemical shift assignments of careaborin did not give in that

report, the tentative chemical shift assignments of this compound, hence, was performed as shown in Table 3.26.

According to all spectral evidences and chemical reactions, it was clearly proved that Compound 12 was careaborin. The structure of this compound is shown below:



Compound 12 Careaborin

3.3.13 Structural Elucidation of Compound 13

Compound 13 as bright white needle crystal, m.p. 229.0-230.0 °C, was obtained from the separation Fraction IE by silica gel column chromatography with 40% chloroform in Fraction No. 30-41. This compound showed a single spot on TLC plate at Rf 0.31 (solvent: chloroform). The colour tests revealed that this compound ought to be an unsaturated triterpenoid since it gave a purple colour to Liebermann-Burchard's and decolourized Br_2 in CCl_4 reagents.

The IR spectrum of this compound (Fig.109) which is assigned in Table 3.28, informed that Compound 13 contained OH as its functional group. The absorption peaks for C-O stretching vibration seem to be different from other isolated triterpenes, i.e.,

it showed two sets of absorption peaks at ν_{max}^{KBr} (cm^{-1}): 1055 and 1045 which could be assigned for C-O stretching vibration of 1° ROH and at 1030 cm^{-1} for C-O stretching vibration of 2° ROH (3 β-OH, probably equatorial). The unsaturation part in this compound also exhibited the vinylidene group ($>C=CH_2$) at 3090, 1645 and 885 cm^{-1} (66,92).

The UV spectrum (Fig.113) gave the λmax at 205 nm. (log ε = 3.64) which hinted non-conjugated system in the molecule.

Table 3.28 The IR absorption band assignments of Compound 13

Frequency (cm^{-1})	Band type	Tentative assignments
3350	very b	O-H stretching vibration
3090	w	C-H stretching vibration of $R_1R_2C=CH_2$
2940,2920 2880	s	C-H stretching vibration of CH_3-, $-CH_2$-
1645	m	C=C stretching vibration
1465,1455	s	C-H bending vibration of CH_3-, $-CH_2$-
1380	s	C-H bending vibration of gem-dimethyl
1055,1045	m	C-O stretching vibration of 1° ROH
1030	s	C-O stretching vibration of 3β -OH (probably equatorial)
885	s	C-H out-of-plane bending vibration of $=CH_2$

From the above evidences, the basic skeleton of this compound, thus, should be composed of terminal methylene group ($R-C=CH_2$), may be two O-H functional groups (one was primary alcohol and the other was secondary one at C-3 position) in a triterpenoidal molecule.

Supported by the mass spectrum data and the elemental analysis result, the structural formular of this compound was proposed to be $C_{30}H_{50}O_2$ (MW. 442.39).

The presence of two hydroxy group (diol) in this compound was proved by the convertion of this compound to its acetyl derivative. In the mass spectrum of the acetate of this compound, the molecular ion peak, M^+, was detected at m/e (% rel. int.) 526.0 (38.59) (Calcd. for $C_{34}H_{54}O_4$: MW. 526.42) which was corresponded to Compound 13 diacetate.

The ^1H NMR of Compound 13 (Fig.111) showed the singlet signal with a relative intensity of 2 protons at 4.91 ppm. characteristic of terminal olefinic hydrogens in agreement with the IR spectrum. The signal at 4.12 ppm. (2H,s) was thought to be due to the protons attaching to electronegative groups. The broad signal at 3.19 ppm. with an integration for 1H was typical for a hydrogen geminal to a hydroxy group at C-3 in usual triterpenoidal skeleton (87). A set of six methyl groups was observed at δ (ppm.): 0.76, 0.78, 0.82, 0.95, 0.96 and 1.03 ppm. (3H each). The ^1H NMR chemical shift assignments of this compound are presented in Table 3.29 (63).

Table 3.29 The ^1H NMR chemical shift assignments of Compound 13

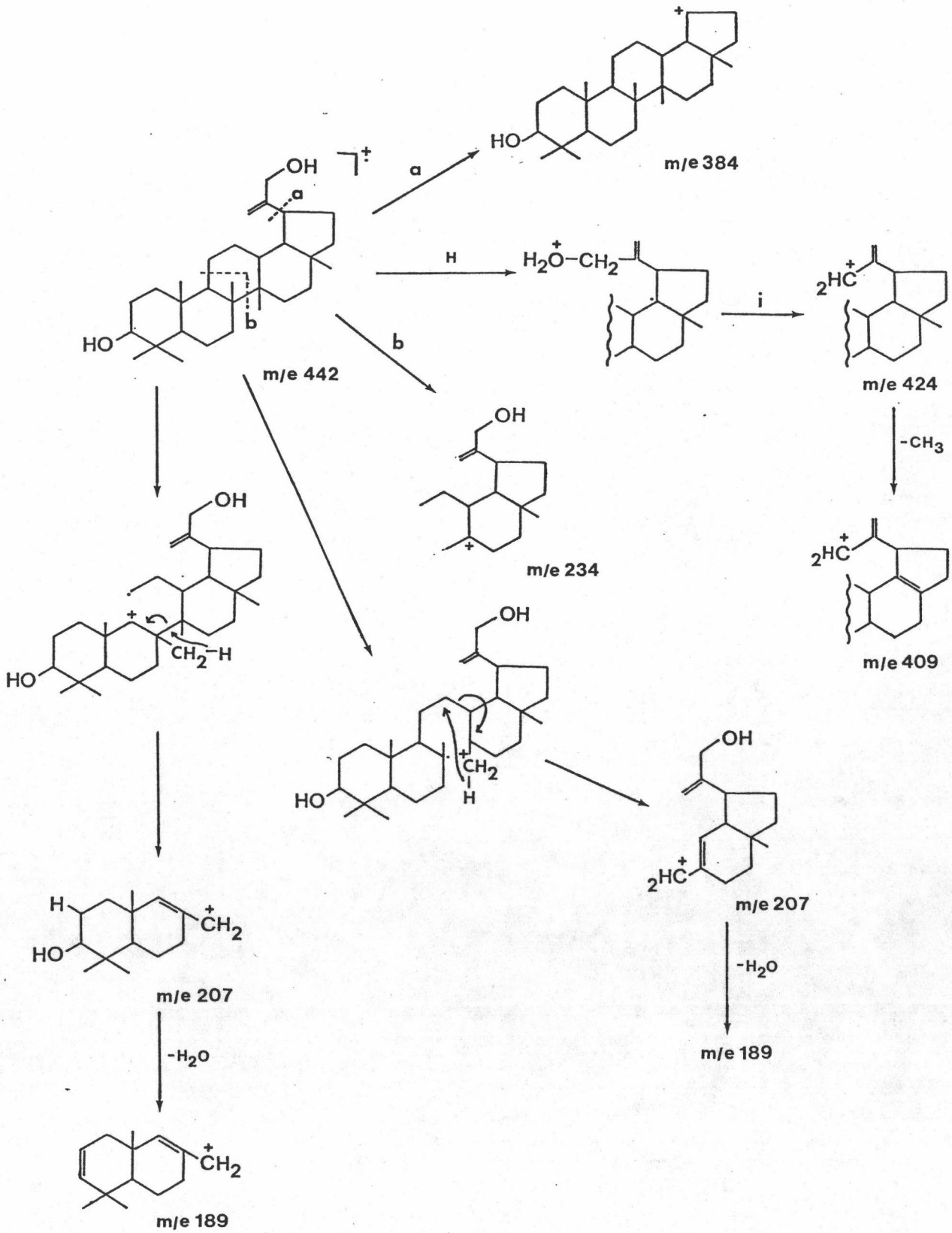
Compound	Chemical shift (ppm.)			
	terminal olefinic protons	carbinol protons		methyl protons
		C-3	C-30	
wallichenol	4.95 (2H,s)	3.25 (1H,dd)	4.15 (2H,s)	0.75,0.78,0.82, 0.93,0.96,1.03 (3H each)
Compound 13	4.91 (2H,s)	3.19 (1H,b)	4.12 (2H,s)	0.76,0.78,0.82, 0.95,0.96,1.03 (3H each)

The mass spectrum of this compound (Fig.110) displayed the parent ion peak at m/e (% rel int.) 442.0 (35.62, M^+) together with a series of fragmentation ion peaks at m/e 207.0 (100.00), 203.0 (42.68) and 189.0 (86.67). This set implied that this triterpenoid should belong to lupane triterpenoid series (73). The lupane series is characterized by contraction of Ring E to five member ring to which an isopropyl or isopropenyl group is attached. The loss of 43 units (C_3H_7) is very pronounced in certain members, but become minimal in high substituted derivatives or in the presence of an isopropenyl functional group. The mass spectrum of Compound 13 did not give the fragmentation ion peaks due to lose 43 unit, however, it displayed dominant fragmentation ion peaks at m/e 424.0 (18.75, M^+-H_2O), 411.0 (5.20, M^+-CH_2OH), 384.0 (15.25, $M^+-CH_2=C-CH_2OH$) instead. The mass spectrum of its diacetate gave the M^+ at 526.0 together with important ion peaks at m/e 466.0 (84.12), 406.0 (8.09) compatible with M^+-OAc, M^+-2OAc, respectively. Other

Other fragmentation ion peaks were similar to those of Compound 13. The possible mass fragmentation pattern of this compound is written in Scheme 3.12.

It was lucid to place 1° ROH in the position next to the terminal methylene at side chain. Owing to the reason that the fragmentation ion peak in the mass spectrum at m/e 384.0 (15.25) due to the loss of $HO-CH_2-\overset{1}{C}=CH_2$ from the parent ion peak and the signal at 4.11 ppm. (2H) in the ^1H NMR spectrum was corresponded to methylene protons attached to a hydroxy group and adjacent to an olefinic system.

Scheme 3.12 The possible mass fragmentation pattern of Compound 13



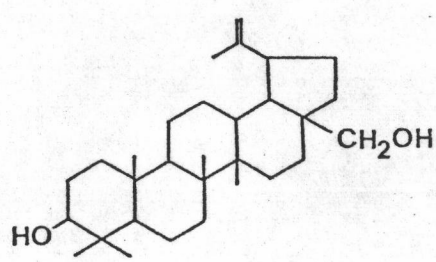
The ^{13}C NMR spectrum of this compound (Fig.112) revealed the carbon signals similar to lupeol (Compound 8), i.e., the terminal olefinic carbons positioned at 154.83 and 106.89 ppm.. The ^{13}C NMR off resonance spectrum of these position revealed that the former carbon should be a skeleton like $>C=$ owing to the presence of a singlet peak, whereas the structure like $=CH_2$ was fixed for the latter because of the splitted triplet peak. The signals at 79.91 and 65.06 ppm. could be assigned for the carbon atoms adjacent to hydroxy group. Like other triterpenoids, the downfield signal was compatible with the C-3 position (98). The signal at 65.06 ppm. was attributed to the carbon bearing hydroxy group in side chain of this lupene triterpenoid. The ^{13}C NMR off resonance spectrum also supported to this observation. It gave a doublet signal at the position 79.91 ppm. due to the CH-OH structure, while the triplet signal centered at 65.06 ppm. was present according to the $-CH_2-OH$ skeleton. Other signals around 55.31 to 14.57 ppm. in both the ^{13}C NMR and the ^{13}C NMR off resonance spectra gave good agreement to methyl, methylene, methine and quarternary carbons. The tentative assignments of the ^{13}C NMR chemical shifts of this compound are presented in Table 3.30.

Table 3.30 The ^{13}C NMR chemical shift assignments of Compound 13

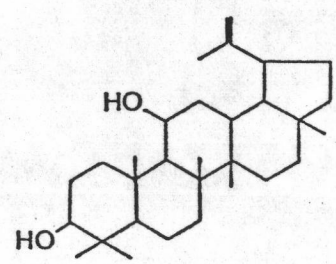
Carbon	Chemical shift (ppm.)	
	wallichenol diacetate	Compound 13
1	38.12	38.73
2	27.46	27.41
3	80.86	78.55
4	38.45	38.90
5	55.42	55.31
6	18.24	18.31
7	34.35	34.35
8	40.95	40.90
9	50.35	50.40
10	37.10	37.00
11	21.11	21.02
12	26.50	26.71
13	37.84	38.03
14	42.84	42.80
15	27.46	27.41
16	35.47	35.48
17	43.02	43.01
18	49.00	48.97
19	44.37	43.83
20	149.29	154.83
21	31.16	31.80
22	39.85	39.87
23	27.96	28.01
24	16.05	15.38
25	16.53	16.14
26	16.17	15.90
27	14.58	14.57
28	17.76	17.72
29	110.16	106.89
30	65.95	65.06
$\underline{CH}_3\text{-}CO_2\text{-}$	20.95	-
$CH_3\text{-}\underline{CO}_2\text{-}$	170.71	-

This lupene diol compound was firstly thought to be betulin ($C_{30}H_{50}O_2$) (I) which widely occurred in mangrove plants. The IR spectrum of both compounds was also coincided. Compound 13, however, was not acceptable to be I according to the m.p. and some spectroscopic data. To illustrate, the m.p. of Compound 13 was 229.0-230.0 °C, while the reported betulin melted at 250.0-253.0 °C (74). Although, almost proton signals in the ^1H NMR spectrum were corresponded to those of betulin, the presence of the proton signal at δ 4.11 ppm. (2H) in Compound 13 was differed from the one that attached on C-28 in betulin (The reported proton signal at C-28 of betulin was at 3.61 ppm.). Compound 13, therefore, was exactly not betulin.

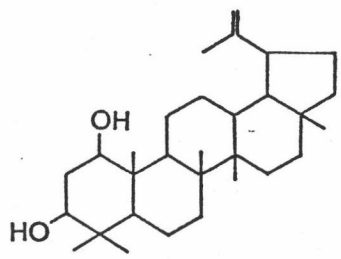
Literature surveys on Lupene-diol triterpenoids were carried out and found that there were not a small number of compounds belonging to this triterpenoidal type. For instance, nepeticin (II), glochidiol (III), thurberis (IV), etc. (121-123). Owing to the mass fragmentation pattern, these compounds gave disagreement with Compound 13.



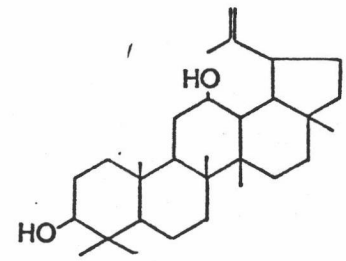
(I)



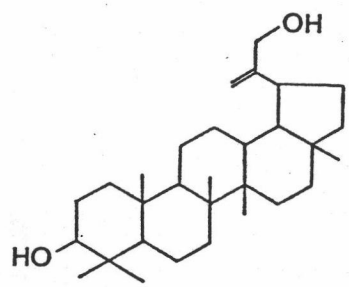
(II)



(III)



(IV)



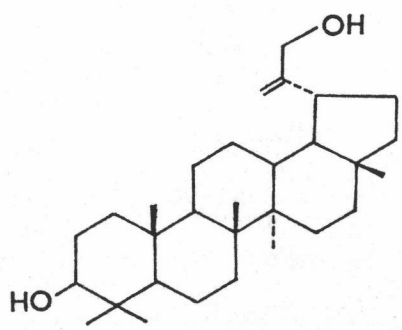
(V)

Considering basing on the mass fragmentation pattern and the m.p. of the lupenediol series, it was found that wallichenol (V), lupan-20(29)-en-3 β ,30-diol, was corresponded to Compound 13 (120,124-125). Since a lack of the authentic sample, the direct mixed m.p., Co. TLC or comparing its spectroscopic data were impossible. Therefore, the best way to ensure that this compound was wallichenol was to prepare its derivative and compare the physical properties and the spectroscopic data with the reported one. The comparision data of the melting point of these two compounds, wallichenol and Compound 13 is given in Table 3.31.

Table 3.31 The comparision data between wallichenol and Compound 13

Compound	Melting point (℃)		Ref.
	alcohol	acetate	
wallichenol	231-233	170-172	120,124-125
Compound 13	229.0-230.0	169.0-171.0	-

According to colour tests, spectroscopic evidences and chemical reactions, Compound 13 was exactly wallichenol. The structure of this compound is shown below:



Compound 13 Wallichenol

3.3.14 Structural Elucidation of Compound 14

Compound 14 was isolated from crude Fraction IF, Fraction IG and chloroform extract crude (Fraction IV) by silica gel column and aluminium oxide column, respectively. After recrystallization with hot ethanol for several times, white amorphous product, 680 mg. (0.45% wt. by wt. of Fraction I), m.p. 260.0 °C (dec.), Rf 0.89 (using 20% methanol in chloroform as a developing solvent), was obtained. The colour tests of this compound indicated the presence of a steroidal compound, unsaturation part and also a carbohydrate moiety, since it gave a deep green colour to Liebermann-Burchard's and gave positive tests to Br_2 in CCl_4 and Molisch's reagents, respectively (55,60-61).

The IR spectrum (Fig.118) which was assigned in Table 3.32, strongly pointed out a O-H stretching vibration at 3420 cm^{-1} (b), C-O stretching vibration of glycosidic linkage at 1075-1020 cm^{-1} (s) and an anomeric axial C-H deformation of β-sugar at 890 cm^{-1} (w) (66,67). The additional bands which were compatible with a trisubstituted olefin and gem-dimethyl group were observed at 1640 (w) and 1380 (m) cm^{-1}, respectively.

The UV spectrum (Fig.119) exhibited a non-conjugated system at the λmax 203 nm. ($\log \varepsilon = 3.97$) (70).

Table 3.32 The IR absorption band assignments of Compound 14

Frequency (cm^{-1})	Band type	Tentative assignments
3420	b	O-H stretching vibration
2920,2880	s	C-H stretching vibration of CH_3-, -CH_2-
1640	w	C=C stretching vibration
1460,1365	m	C-H bending vibration of CH_3-, -CH_2-
1380	m	C-H bending vibration of gem-dimethyl gr.
1075-1020	s	C-O stretching vibration of glycosidic linkage
890	w	anomeric axial C-H deformation of β-sugar

The ^1H NMR spectrum (Fig.120) revealed the signals around 2.43 to 0.66 ppm. typical for those of steroid (see also the ^1H NMR spectrum of Compound 10 (Fig.87)). The proton signal at 5.32 ppm. should be an olefinic proton. The rest signals between 4.79 to 3.73 ppm. were assigned for the protons on sugar moiety. The doublet signal at 4.18 ppm. (J=8.00 Hz) which was attributed to an anomeric proton was significant to state the presence of glycosidic linkage. This information was coincided with the IR spectrum and the colour tests that this compound should be composed of a steroidal part adjacent to a sugar moiety at C-3 position with glycosidic linkage (126).

The ^{13}C NMR spectrum (Fig.121) gave the two olefinic carbon signals at 138.71 and 119.21 ppm. and other six carbon

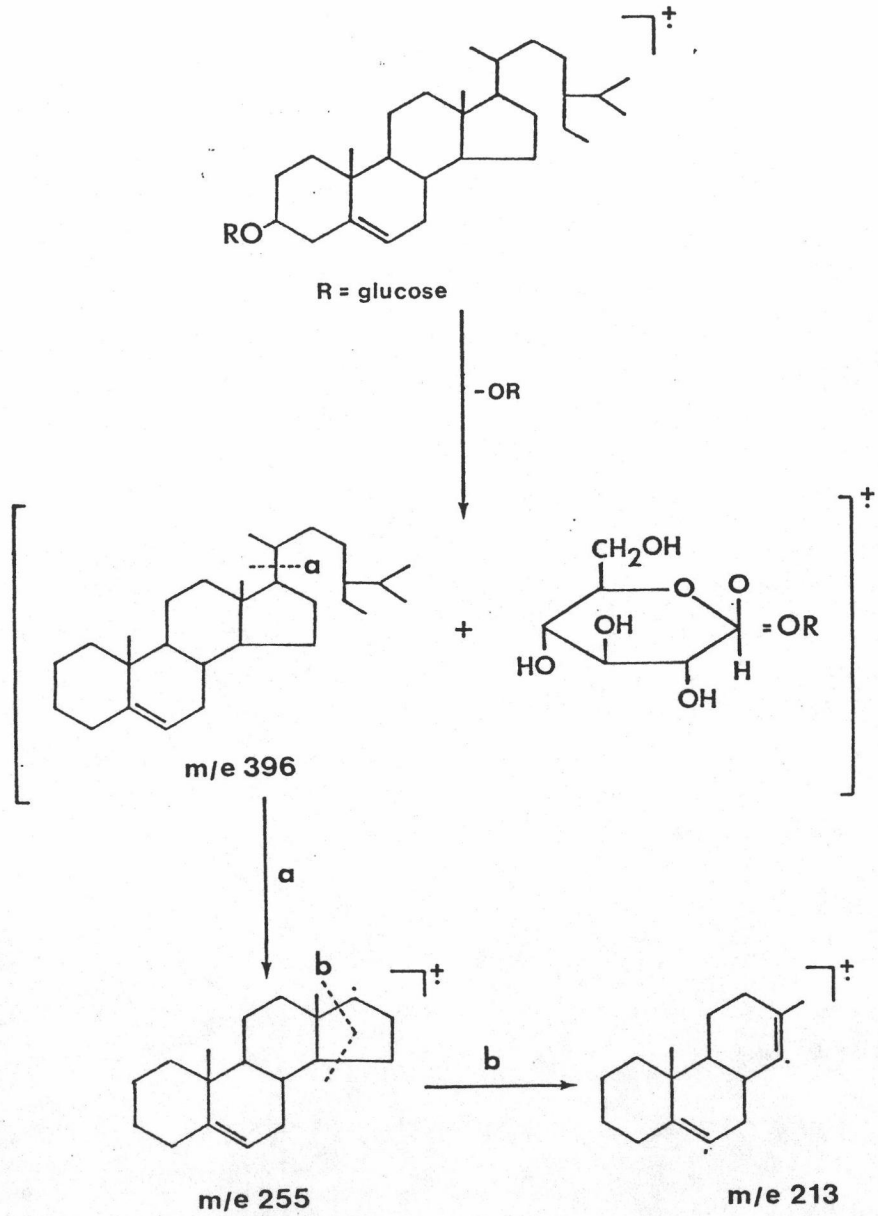
signals of sugar molecule which were observed at 100.25, 78.36, 76.36, 75.81, 72.78 and 69.53 ppm. These signals revealed that this sugar should be a member of hexose group. Some parts of the carbon signals of this compound found to be closely resemble to those of steroidal compounds. The ^{13}C NMR chemical shift tentative assignments of Compound 14 are given in Table 3.33 (127).

The mass spectrum (Fig.122) did not give the molecular ion peak (M^+), but it exhibited the dominant fragmentation ion peaks at m/e (% rel. int.) 414.0 (49.77), 396.0 (100.00, 414-H_2O), 381.1 (27.55, 414-H_2O-CH_3), 273.0 (18.07, 414-$C_{10}H_{21}$), 255.0 (39.74, 414-$C_{10}H_{21}$-H_2O), 213.0 (32.22, 414-$C_{10}H_{21}$-42). From the fragmentation pattern in the mass spectrum suggested that Compound 14 had a steroidal nucleus and a sugar moiety linked together as a steroidal glycoside molecule (128-129). Moreover, the mass fragmentation pattern also indicated the presence of C_{29} steroid compound which was corresponded to β-sitosterol (111). The possible mass fragmentation pattern is given in Scheme 3.13.

Table 3.33 The ^{13}C NMR chemical shift assignments of Compound 14 and Compound 14 acetate

Carbon	Chemical shift (ppm.)			
	β-sitosterol	β-sitosteryl-glucoside	Compound 14	Compound 14 acetate
1	37.25	37.6	37.75	37.22
2	31.66	30.3	30.04	31.91
3	71.77	78.5	78.36	80.07
4	45.84	39.3	44.02	45.08
5	140.72	140.9	138.71	140.37
6	121.64	121.9	119.21	122.16
7	32.89	32.1	32.15	31.91
8	32.89	32.1	32.15	31.91
9	50.14	50.4	53.25	50.16
10	36.51	37.0	37.14	36.73
11	19.39	21.4	21.32	19.34
12	39.82	40.0	40.44	39.76
13	45.84	42.5	48.30	45.08
14	56.75	56.9	54.20	56.77
15	24.29	24.6	24.35	24.32
16	39.76	28.5	27.97	39.76
17	56.07	56.3	54.20	56.77
18	11.97	12.0	10.16	11.86
19	19.04	19.4	17.96	19.07
20	33.95	36.4	27.38	33.97
21	18.78	19.2	17.42	18.80
22	36.15	34.3	34.27	36.13
23	23.07	26.5	22.46	23.08
24	42.31	46.1	44.02	42.36
25	28.24	29.5	27.38	28.22
26	21.10	19.4	17.96	21.07
27	19.80	20.0	19.26	19.83
28	26.11	23.5	26.41	26.11
29	11.86	12.2	10.00	11.86
C-1'	-	102.6	100.25	99.68
C-2'	-	75.3	75.43	72.92
C-3'	-	78.5	75.81	71.73
C-4'	-	71.7	72.78	71.51
C-5'	-	78.5	75.81	68.58
C-6'	-	62.8	60.70	32.14
$\underline{CH_3}$-COO-	-	-	-	20.64,20.75 29.20,29.47
CH_3-$\underline{COO}$-	-	-	-	173.41,170.70 170.40,169.40

Scheme 3.13 The possible mass fragmentation pattern of Compound 14



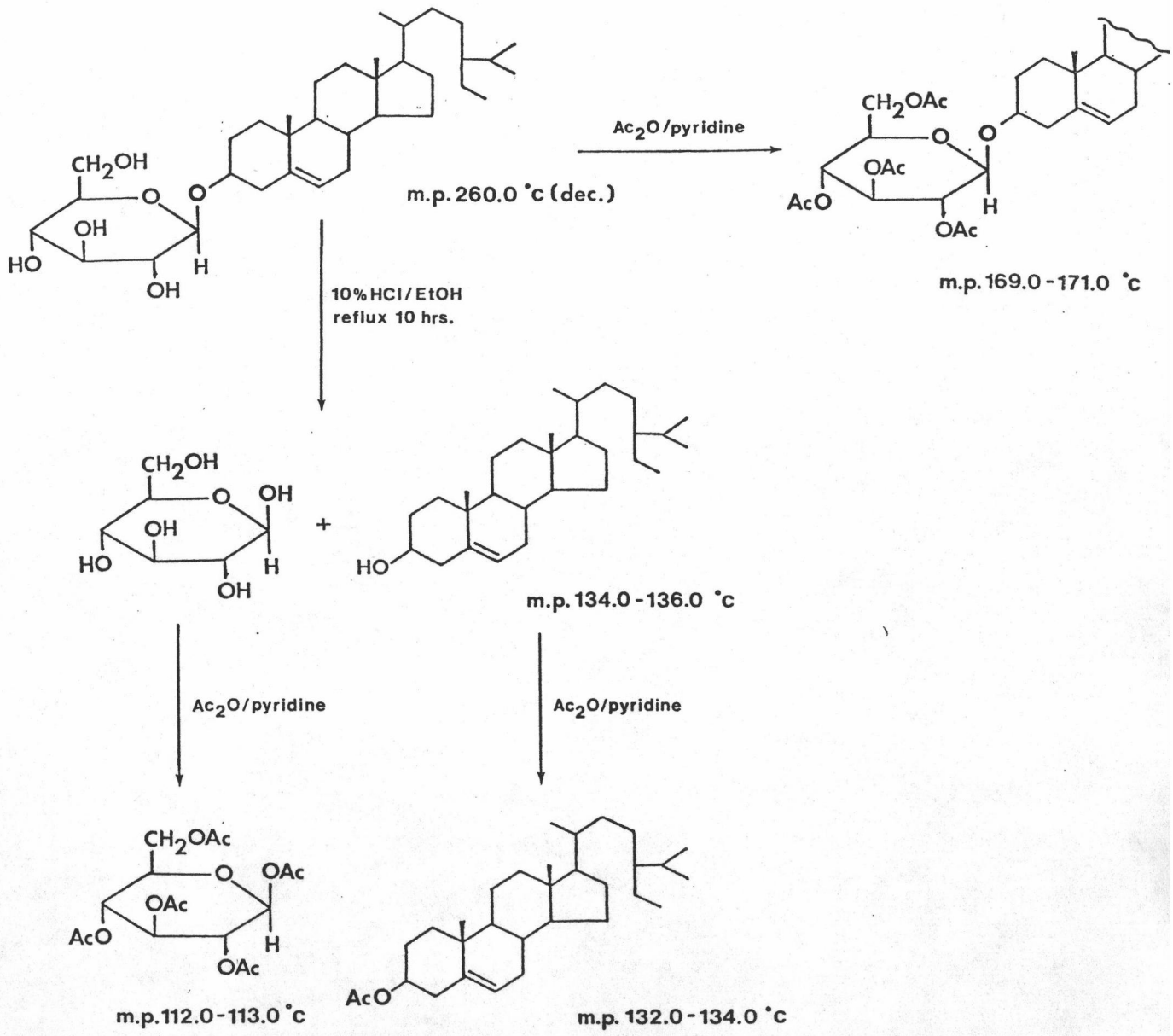
To settle the structure of this compound, the acid hydrolysis reaction of Compound 14 with 10% HCl in ethanol was carried out and gave an aglycone part, designated as Compound 14A and a glycone fraction, labelled as Compound 14B.

The aglycone part, Compound 14A, gave good agreement to β-sitosterol by mixed m.p., Co. TLC and the GLC analysis. Moreover, the acetyl derivative of Compound 14A was synthesized and found that Compound 14A acetate had to be β-sitosterylacetate due to the results from mixed m.p., Co. TLC and comparing its IR spectrum with the authentic one.

The glycone part, Compound 14B, was examined by various ways such as PC, TLC, HPLC and found that it was corresponded to D-glucose. Beside using chromatographic method, the acetyl derivative of this compound was prepared and found that its m.p. was close to the reported D-glucosepentaacetate (74).

By means of chemical reactions, Compound 14 was proved to be the glycoside of β-sitosterol and D-glucose. The chemical reactions of this compound are shown in Scheme 3.14.

Scheme 3.14 The chemical reactions of Compound 14



Peracetylated derivative of Compound 14 was synthesized as white amorphous product, m.p. 169.0-171.0 °C, Rf 0.64, using 5% chloroform in methanol as solvent system.

The IR spectrum (Fig.128) revealed the presence of acetyl group at 1759 (-C-) and 1250-1220 (C-O) of acetate. The remainder of this spectrum was similar to the IR spectrum of Compound 14 except the O-H peak was absent.

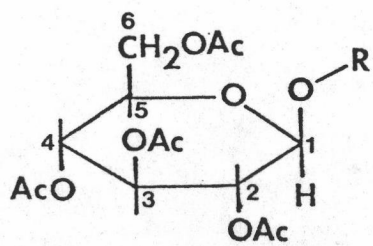
The ^{13}C NMR spectrum (Fig.130) showed the signals of four carboxyl of acetoxy groups at 173.41, 170.70, 170.40 and 169.40 ppm. (1C each), two additional signals belonging to the olefinic protons at 140.37 and 122.16 ppm.. The six carbons of a sugar moiety were observed at 99.68, 72.92, 71.73, 71.51, 68.58 and 62.14 ppm. which were assigned for C_1', C_2', C_3', C_4', C_5' and C_6', respectively (130). The signal at 80.07 ppm. was compatible with the C-3 of steroid attaching to oxygen of sugar molecule. The rest signals were corresponded to those of β-sitosterol. The ^{13}C NMR chemical shift assignments of this compound are given in Table 3.33 (127).

The mass spectrum of this compound (Fig.131) gave M^+ at m/e 744.0 (by mass spectrum, this molecular ion was very weak and detected with difficulty) which was agreed with the molecular formular $C_{43}H_{68}O_{10}$. The fragment of acetoxy group step by step was observed at m/e 684.0, 624.0 and 564.0. The series of fragmentation ion peaks at m/e 331.0, 229.0, 221.0, 169.0 and 109.0 were the characteristic for glycoside tetraacetate (131). Other fragmentation ion peaks were found to be corresponded to β - sitosterol (111).

The ^{1}H NMR spectrum of the acetate of this compound (Fig.129) gave completely coincided with that of the reported β-sitosteryl-3-O- β-D-glucopyranosidetetraacetate (120), i.e., it revealed seven signals belonging to the protons on sugar moiety which is tabulated below.

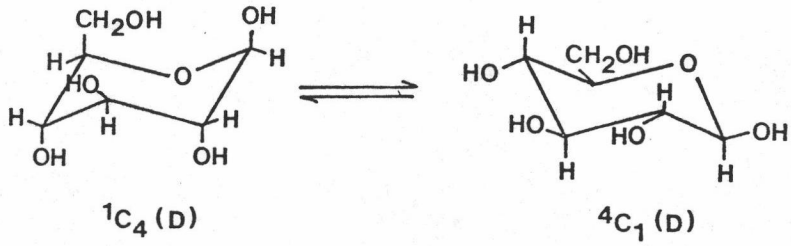
Table 3.34 The ^{1}H NMR chemical shift assignments of sugar moiety of Compound 14 tetraacetate

Compound	Chemical shift (ppm.)						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6'
β-sitosteryl-3-O-β-D-glucopyranoside tetraacetate	4.59	4.93	5.21	5.06	3.68	4.27	4.13
Compound 14 tetraacetate	4.54	4.96	5.15	5.06	3.69	4.25	4.19



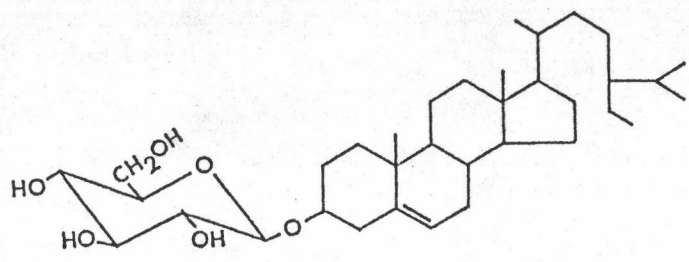
The olefinic proton at C5-6 of β-sitosterol occurred at 5.39 ppm. (1H), the protons of 4 acetoxy groups (-O-C(=O)-CH$_3$) were observed at 2.08, 2.05 and 2.02 ppm. with integration equal to 12H. The anomeric protons of sugar at 4.58 ppm.(1H,d,J=7.81 Hz) indicated the β-glycosidic linkage (133-134). The β-glycoside linkage has two

possible chair conformations as $^{4}C_{1}$ (D): J=7-10 Hz and $^{1}C_{4}$ (D): J=2-4 Hz). These two conformations are differed at an anomeric proton and proton at C-2 which are diaxial and equatorial, respectively. The observed value of coupling constant (J=7.81 Hz) was in good agreement with $^{4}C_{1}$ (D) chair conformation of β-D-glucopyranoside (134). The two possible chair conformations of β-D-glucopyranoside are shown below:



$^{1}C_{4}$ (D) $^{4}C_{1}$ (D)

From spectral evidences and some chemical reactions confirmed that a non-aqueous part was β-sitosterol, and an aqueous part was β-D-glucopyranose. Peracetylation of Compound 14 clearly suggested that the glucose unit in the glycoside was β-D-glucopyranose which was attached at C-3 of β-sitosterol. According to the information above, Compound 14, hence, was clearly to draw its structure as β-sitosteryl-3-O-β-D-glucopyranoside. The structure of this compound is shown below:



Compound 14 β-sitosteryl-3-O-β-D-glucopyranoside

3.3.15 Structural Elucidation of Compound A

Compound A was obtained from the separation of Fraction IE by silica gel column chromatography in Fraction No. 63-89. After recrystallization this compound with a large amount of chloroform and methanol, Compound A as white amorphous solid, 10 mg. (0.0067% wt. by wt. of Fraction I), m.p. 280.0-283.0 °C (dec,) was obtained. This compound gave only one spot at Rf 0.61 using 2% methanol in chloroform as mobile phase. Compound A gave a violet colour to Liebermann-Burchard's, decolourized Br_2 in CCl_4 and also gave a positive test to Molisch's reagents. This information implied that Compound A should contain a triterpenoidal skeleton, unsaturation part and carbohydrate moiety (55,60-61).

The IR spectrum (Fig.116) informed that this compound contained O-H (at 3430 cm^{-1}) as its functional group together with unsaturation part and aromatic moiety (3050 (C-H of alkene or aromatic), 1620,1520,1450 (C=C) and 830,820 (C-H out-of-plane). Moreover, an ester linkage (1720 (-$\overset{O}{\overset{\|}{C}}$-O-) and 1250, 1170 (C-O)) and glycosidic linkage (1020 (C-O)) were also presented in this molecule. The IR absorption band assignments of Compound A are shown in Table 3.35 (66,67).

The information obtained from the IR spectrum and the colour tests suggested that this compound should be triterpenoidal compound joined to other molecules (may be aromatic compound and sugar moiety) by ester linkage and glycosidic bond.

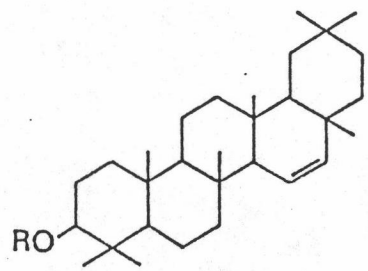
Table 3.35 The IR absorption band assignments of Compound A

Frequency (cm^{-1})	Band type	Tentative assignments
3430	b	O-H stretching vibration
3050	w	C-H stretching vibration of $R_1R_2C{=}CR_3H$ or of aromatic skeleton
2950,2850	s	C-H stretching vibration of CH_3-,-CH_2-
1720	s	-C(=O)- stretching vibration of ester
1620,1520 1450	m	C=C stretching vibration of alkene or of aromatic skeleton
1450,1390	m	C-H bending vibration of CH_3-,-CH_2-
1380	m	C-H bending vibration of gem-dimethyl group
1250,1170	s	C-O stretching vibration of ester
1020	s	C-O stretching vibration of glycosidic linkage
830,820	w	C-H out-of-plane of alkene or aromatic skeleton

The mass spectrum of this compound did not give the molecular ion peak, but it displayed the dominant fragmentation ion peaks at m/e (% rel int.) 426.0 (3.33), 408.0 (19.61, 426-H_2O), 284.0 (31.27), 269.0 (47.05), 204.0 (100.00) and 189.0 (47.06). The series of the dominant fragmentation ion peaks at m/e 426.0, 408.0, 284.0, 269.0, 204.0 and 189.0 was resemble to that of taraxerol

triterpenoid and its related compounds (see also Compound 9, 11 and 12) (73). Hence, it was reasonable to propose that the triterpenoid in this compound may be taraxerol.

Unfortunately, this compound was obtained in such a limit amount that its structure could not be completely elucidated. However, some part of this substance could be proposed as follows:



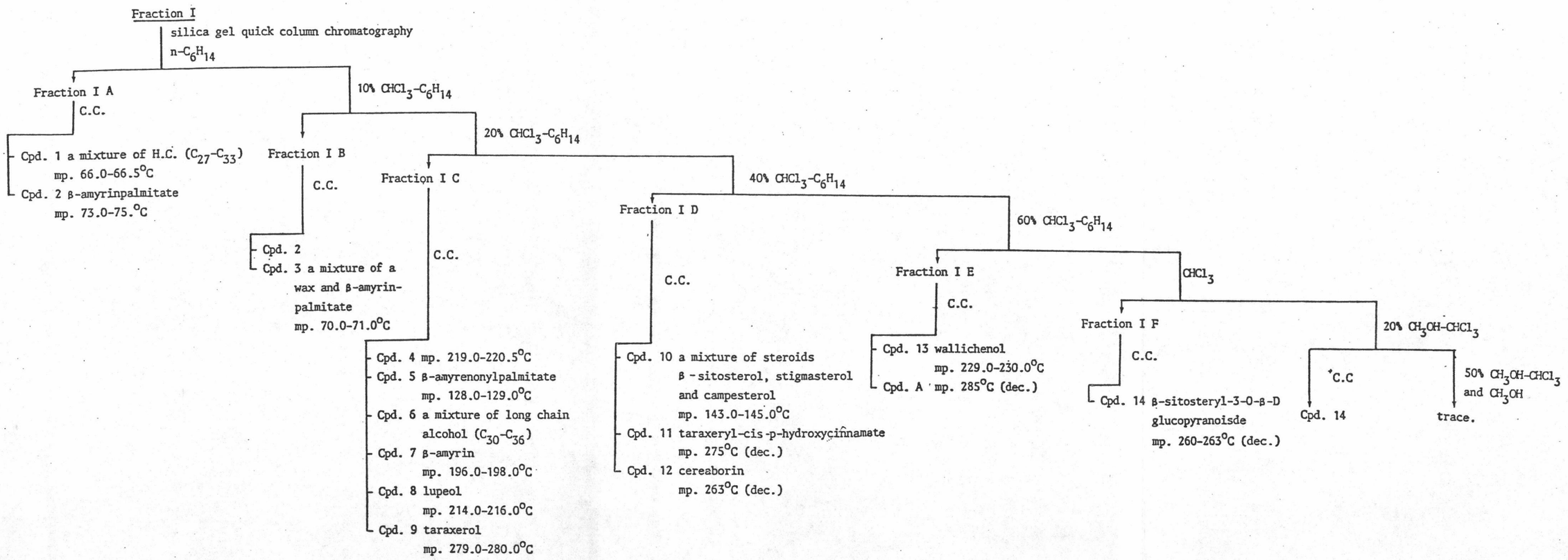
Compound A

In case of inadequate compound, the hydrolysis reaction was useful to elucidate this kind of substance. Since the ester linkage and glycosidic one were hydrolyzed in different condition, i.e., for acidic medium the glycosidic linkage was easily hydrolysed, while in the alkaline solution, the ester bond was easily damaged (63).

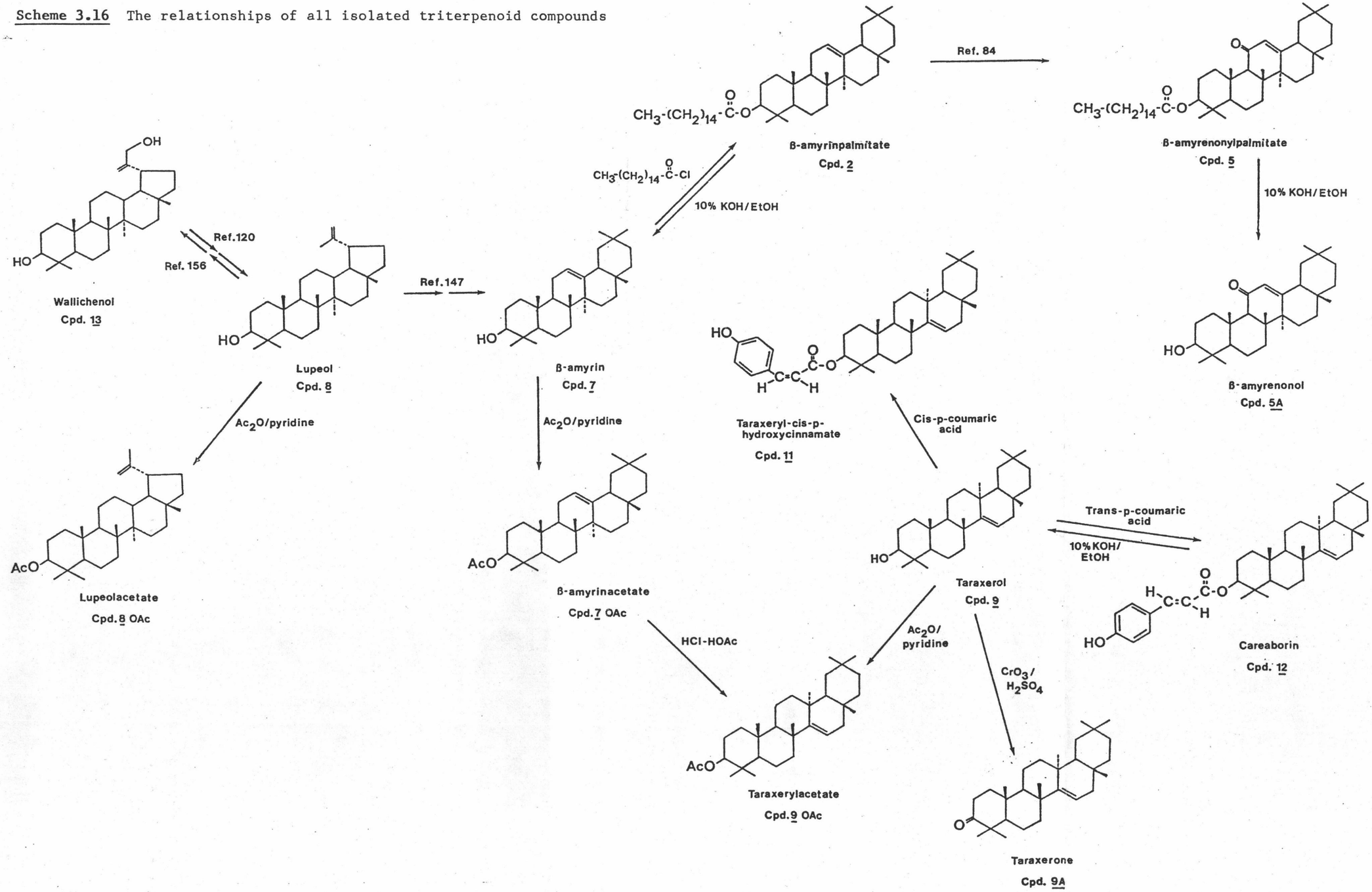
All the isolated compounds separated from Fraction I is summarized and presented in Scheme 3.15.

Throughout the chemical literatures, all isolated triterpenoids from the leaves of R. apiculata were found to have close relationships which could be summarized as shown in Scheme 3.16.

Scheme 3.15 The conclusion of isolated compounds from Fraction I



Scheme 3.16 The relationships of all isolated triterpenoid compounds



3.4 Chemical Constituents of Fraction II

Fraction II, as a white solid 50.50 g. (0.78% wt. by wt. of dried plant material), was separated during the removal the solvent from the 95% ethanol soluble fraction. Ignition tests found that this fraction was an inorganic salt fraction. Qualitative analysis informed that this fraction was mainly chloride salts of sodium, potassium and magnesium. The quantitative analysis of the cation composition by FES found that the major components were sodium and potassium 59.60% and 40.10%, respectively, whereas magnesium was present approximately 0.30%.

3.5 Chemical Constituents of Fraction III

Fraction III as a brown-reddish solid 135.80 g. (2.09% wt. by wt. of dried plant material), was insoluble in both chloroform and water. This fraction was preliminary screened by chemical reagents and phytochemical methods and found that Fraction III was a condensed and hydrolysable tannin fraction. It is well-known that the hydrolysable tannins can be hydrolyzed by acids or enzymes to simple units, while the condensed ones are converted into more complex, insoluble, coloured products called "phlobophens" (89).

To verify that Fraction III contained hydrolysable tannin, this fraction was hydrolyzed by 20% HCl in ethanol by refluxing for 10 hours. The entire reaction mixture was, then, concentrated, added 30.0 mL. of water and extracted with ethylacetate. The ethylacetate soluble fraction was studied further by using HPLC. The results from the HPLC analysis concided with the presence of gallic acid. Therefore, the hydrolysable tannin in Fraction III

ought to belong to a gallotannin class.

In conclusion, Fraction III was mainly composed of condened and hydrolysable tannins. The latter was proved to be a member of gallotannin.

3.6 Chemical Constituents of Fraction IV

Fraction IV, chloroform soluble fraction 105.00 g. (1.22% wt. by wt. of dried plant material), was obtained by equilibrating the ethanolic extract with chloroform and water (1:1). The separation of this fraction by aluminium oxide column chromatography gave only one crystalline compound. After purification by recrystallization with hot ethanol for several times, a white amorphous substance 500 mg. (0.83% wt. by wt. of Fraction IV), m.p. 260.0-263.0 °C (dec.), Rf 0.89 (solvent: 20% methanol in chloroform) was gained. By means of mixed m.p., Co. TLC techniques and comparing its IR spectrum with Compound 14, this compound was, undoubtedly, found to be indentical to Compound 14.

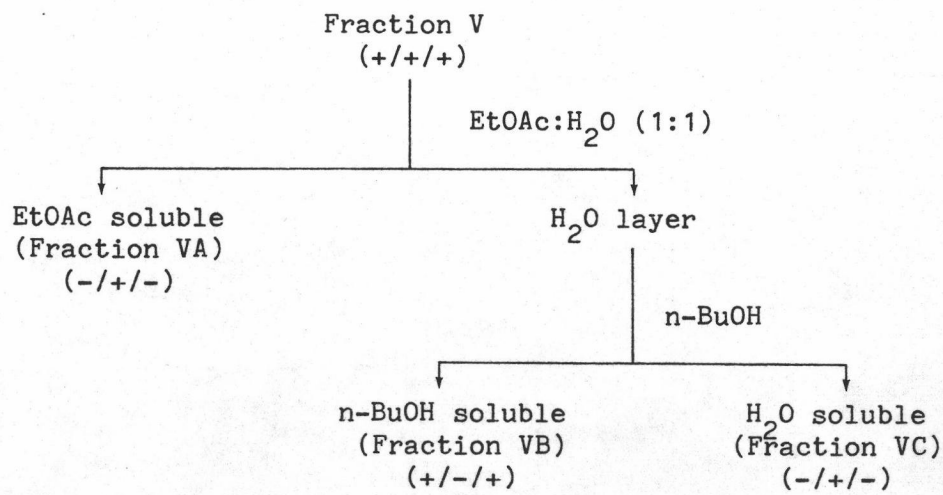
Thus, the only one crystalline product contained in this fraction was β-sitosteryl-3-O-β-D-glucopyranoside.

3.7 Chemical Constituents of Fraction V

Fraction V as a reddish syrup 145.10 g. (2.33% wt. by wt. of dried plant material) was derived from the extraction of the aqueous layer with n-butanol. To examine this fraction, the phytochemical screening tests were conducted and found that this fraction revealed the presence of flavone compounds, phenolic substances and carbohydrate moiety. (It gave a red colour like bird's blood to

Shinoda's, a deep green colour to 5% $FeCl_3$ and showed a positive test to Molisch's reagents). This result, especially for the presence of flavone compounds, was corresponded to preliminary screening tests, i.e, the ethanolic extract of the leaves of this plant showed the possibility to contain flavone compounds (see also Table 2.2). The further extraction of this fraction was carried out and yielded ethylacetate soluble fraction (Fraction VA), n-butanolic soluble fraction (Fraction VB) and water layer (Fraction VC). These three divided fractions were reexamined with Shinoda's, 5% $FeCl_3$ and Molisch's reagents. The results of this examination are summarized as shown in Scheme 3.17.

Scheme 3.17 The results of examination for Fraction V



Note (+/+/+) stands for positive tests to Shinoda's, $FeCl_3$ and Molisch's reagents, respectively.

(-/-/-) stands for negative results.

From Scheme 3.17, it was found that the phenolic compounds were present in ethylacetate fraction, whereas the flavone substances existed in n-butanolic fraction. The presence of carbohydrate moiety (may be sugar or glycoside) were in both n-butanolic and water soluble fraction.

Due to a limit of time and a lack of appropriate adsorbents and instrumentation for separation the polar parts, the two latter fraction, Fraction VB and VC, have not been studied.

The ethylacetate fraction (Fraction VA) which revealed a deep green colour to 5% $FeCl_3$ reagent should contain phenolic compounds. The HPLC analysis was selected to examine this fraction further, however, this kind of analysis needs the authentic samples. The authentic samples used in this examination were the phenols and their related compounds* which were reported to be widely distributed in plants. For example, gallic acid, cinnamic acid, pyrogallol, vanillin, phloroglucinol, catechol, etc.

The results from the HPLC analysis found that Fraction VA ought to contain gallic acid, cinnamic acid, p-coumaric acid and may be present of resorcinol, phloroglucinol, benzoic acid, syringaldehyde, p-hydroxybenzoic acid, vanillin and m-hydroxy-benzoic acid. The retention times of the authentic sampls are given in Table 3.36, their structures are drawn in Fig.192 and the

---

* Phenol and their related compounds are one of the most widely distributed substances in natural products, nevertheless, the free phenols were found relatively rare in plants rather than phenolic carboxylic acids (108).

results of investigation phenolic compounds compared with the retention time of the authentic ones are shown in Table 3.37.

Table 3.36 The retention times of the authentic phenolic compounds

Substances	Rt (min.)	Substances	Rt (min.)
gallic acid	3.27	m-hydroxybenzoic acid	12.07
pyrogallol	3.51	vanillin	14.12
hydroquinone	3.54	p-coumaric acid	17.99
resorcinol	5.25	tannic acid	21.31
catechol	6.94	benzoic acid	22.44
p-hydroxybenzoic acid	9.67	salicylic acid	25.86
syringaldehyde	10.00	phthalic acid	28.12
phloroglucinol	10.25	cinnamic acid	33.06

Table 3.37 The HPLC analysis results of Fractoin VA

Rt (min.)	Indication	Rt (min.)	Indication
3.27	gallic acid	25.50	
4.82		25.93	
4.58	(resorcinol)	26.33	
6.05		27.95	
9.90	(p-hydroxybenzoic acid)	28.97	
10.17	(syringaldehyde, phloroglucinol)	29.80	
		30.70	
12.18	(m-hydroxybenzoic acid)	31.40	
13.80	(vanillin)	32.97	
17.97	p-coumaric acid	34.20	cinnamic acid
21.57	(tannic acid)	34.67	
23.00	(benzoic acid)	35.20	
24.07		35.77	

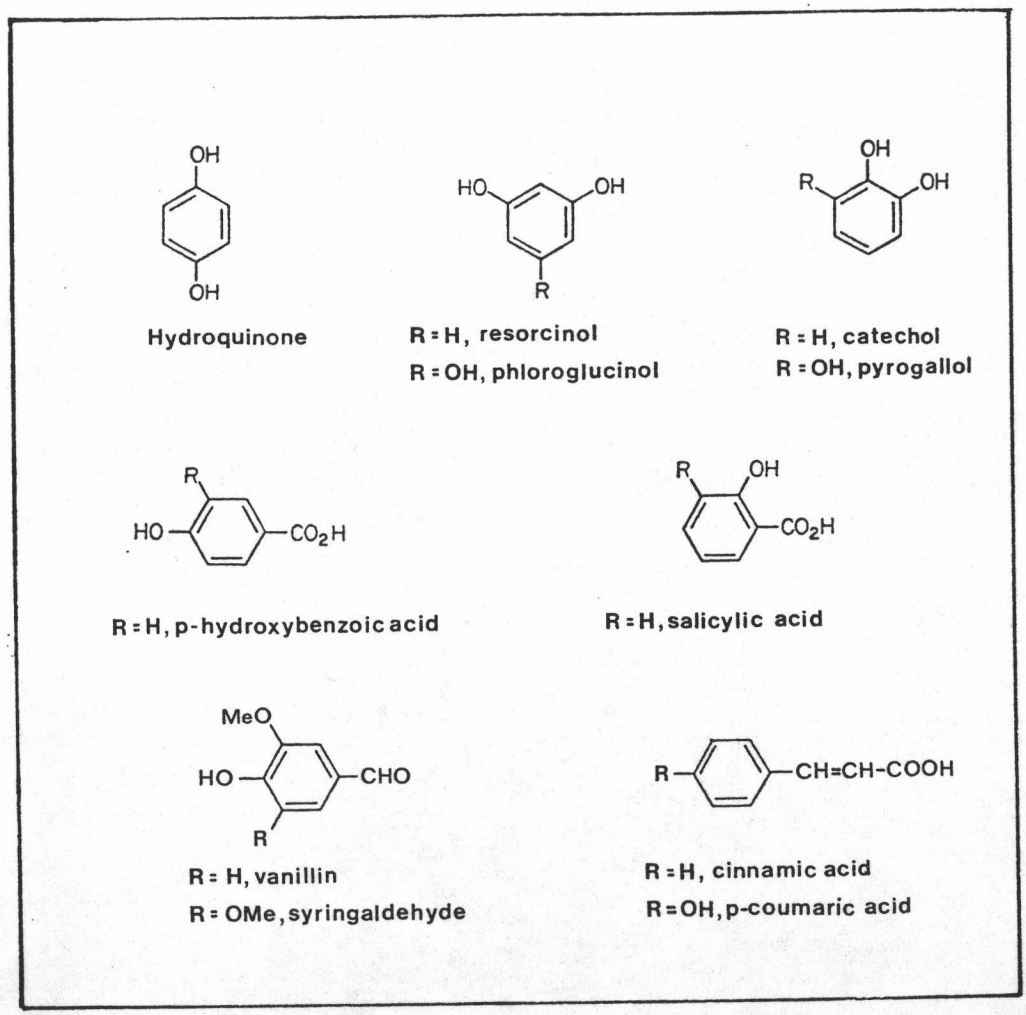


Figure 192 The phenolic and its related compounds used as the authentic samples for HPLC analysis

This fraction was studied further according to the publication of S.N. Ganguly and S.M. Sirger who had reported to isolate some gibberellins from some kinds of mangrove plants in ethylacetate soluble fraction. This fraction, hence, was screened for gibberellins* by using HPLC. The results revealed that Fraction VA may containe gibberellin A_3, but not A_4 and A_7. The structure of gibberellin A_3 is presented in Fig.6.

In case of available adsorbent and instrumentation, the n-butanolic extract crude of R. apiculata is worth investigation, since the preliminary bioassay experiments of this fraction indicated a strong inhibition against the insect, boll weevil.

3.8 Chemical Constituents of Fraction VI

Fraction VI, the freeze-dried water soluble fraction as a reddish syrup, 270.43 g. (4.16% wt. by wt. of plant material) was further extracted into methanol soluble fraction (Fraction VIA) and methanol insoluble fraction (Fraction VIB) (see also Scheme 2.8).

Study on Fraction VIA

From the preliminary tests of Fraction VIA revealed that this fraction should contain carbohydrates (reducing sugar) and amino acids. The examination on carbohydrate components were carried out in various methods such as PC, HPLC (by comparision with the

---

* The authentic gibberellin A_3, A_4 and A_7 are kindly supplied by Miss Wanrudee Nimchareounwongsa, Department of Biotechnology and Chemical Engineering, Chulalongkorn University.

standard sugars*) and conversion into the osazone and acetyl derivatives. The results from the above analysis were coincided with each other and could be concluded that the major sugar component in Fraction VIA was arabinose. The investigation on amino acids in this fraction was performed by using Amino Acid Analyser and found that there were several amino acids contained in this fraction. The results of both qualitative and quantitative analyses for amino acids of Fraction VIA are presented in Table 3.38.

Table 3.38 The qualitative and quantitative analysis for amino acids of Fraction VIA

Amino acids	Conc[a]	Amino acids	Conc[a]
aspartic	0.20	leucine	0.08
threonine	0.27	tyrosine	0.22
serine	0.61	β-alanine	0.39
glutamic	0.12	γ-aminobutyric	3.69
glycine	0.21	ethanolamine	-
alanine	5.39	ammonia	48.30
valine	2.73	lysine	0.04
methionine	0.39	arginine	0.35
isoleucine	0.31	proline (440)	-

[a] The concentration of amino acids is calculated in mole amino acid/ 20 mg. of crude Fraction VIA.

* The standard sugars are rhamnose, arabinose, glucose, galactose and xylose.

Study on Fraction VIB

Fraction VIB was found to be an inorganic salt fraction by chemical and ignition tests. The preliminary qualitative analysis revealed that this fraction ought to be chloride salts of Na, K and Mg. The X-ray fluoresence data (Fig.138) indicated the presence of Na, K, Mn and Cl. The quantitative analysis for the cations contained in this fraction by ICPS found that the major constituents in Fraction VIB was Na, K and Mg 55.00%, 27.96% and 14.38%, respectively together with Ca 1.88% and Mn 0.70%. The Fe and Cu were occurred as trace elements in this fraction.

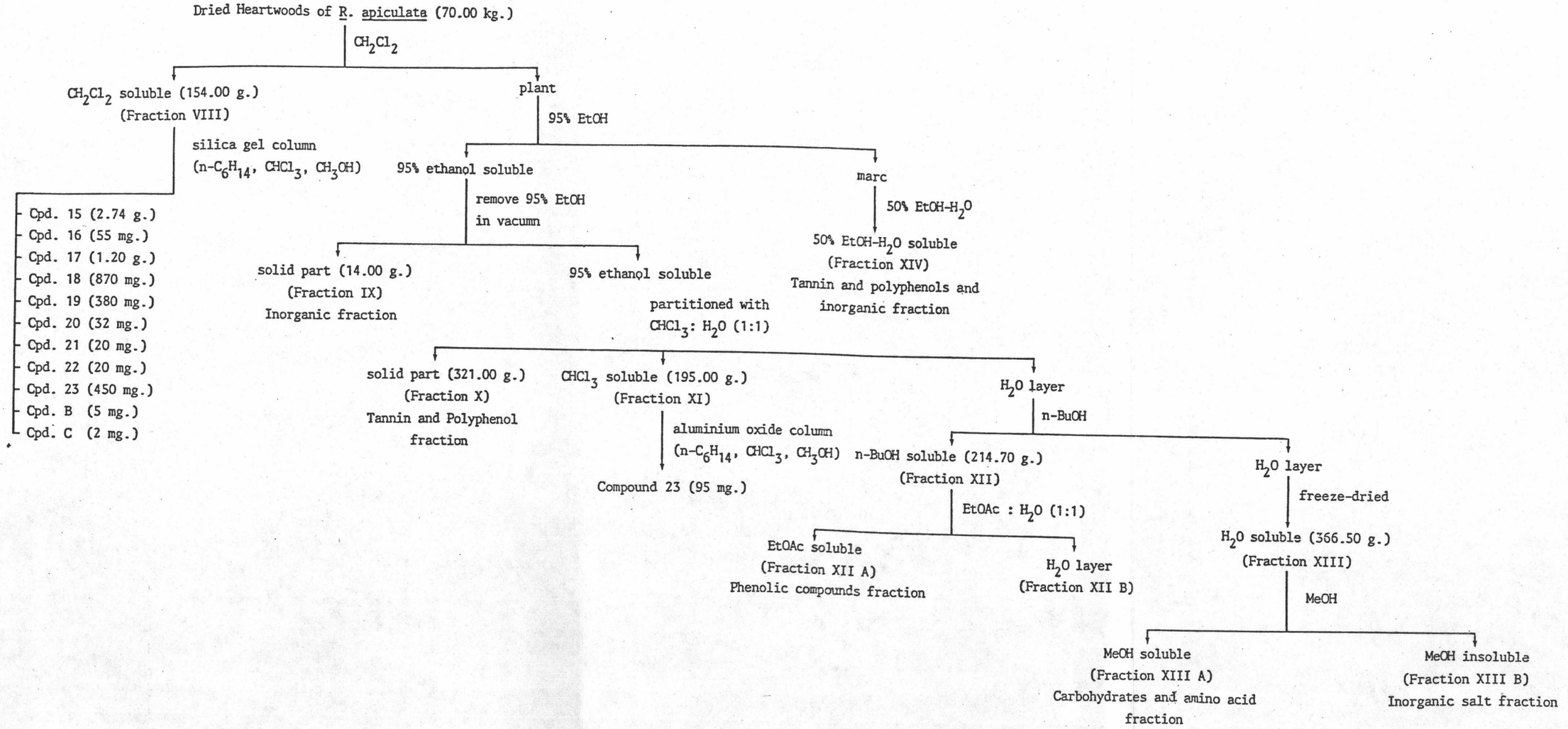
3.9 Chemical Constituents of Fraction VII

Fraction VII was assigned for the leaves after extraction with dichloromethane and 95% ethanol, successively. This fraction was found to be consisted of condensed and hydrolysable tannins as well as inorganic salts. The composition of cations contained in Fraction VII was analysed by ICPS and found that Na, K and Mg were present as major elements, 35.76%, 34.49% and 21.04%, respectively together with Ca 2.92% and Mn 2.76%. The Fe and Cu were trace elements in this fraction, 0.00010% and 0.00016%, respectively.

Part II Chemical Constituents of the Heartwoods of R. apiculata Bl.

The extraction of the heartwoods of R. apiculata which was carried out in the same way as that for the leaves yielded dichloromethane soluble fraction (Fraction VIII, 0.22% wt. by wt. of dried plant material), white solid part (Fraction IX, 0.02% wt. by wt.), solid part (Fraction X, 0.45% wt. by wt.), chloroform soluble fraction (Fraction XI, 0.28% wt. by wt.), n-butanolic soluble fraction (Fraction XII, 0.52% wt. by wt.) and water soluble fraction (Fraction XIII, 0.52% wt. by wt.). The exhausted heartwoods were assigned as Fraction XIV (see also Scheme 2.2). The procedure for extraction and isolation was presented in Scheme 3.18.

Scheme 3.18 The procedure for extraction and isolation Fraction VIII



3.10 Chemical Constituents of Fraction VIII

The extraction of the heartwoods of R. apiculata with dichloromethane yielded a dichloromethane soluble fraction as a yellow-brownish crude 154.00 g. (0.22% wt. by wt. of dried plant material).

The results of separation and purification of crude Fraction VIII by using silica gel column chromatography gave eleven crystalline solid which were marked as Cpd.15 m.p. 85.0-87.0 °C (0.0039% wt. by wt.*), Cpd.16 m.p. 78.0-79.0 °C (0.000078% wt. by wt.*), Cpd.17 m.p. 76.5-78.0 °C (0.0017% wt. by wt.*), Cpd.18 m.p. 139.0-141.0 °C (0.012% wt. by wt.*), Cpd.19 m.p. 230 °C (sublimation) (0.00054% wt. by wt.*), Cpd.20 m.p. 119.0-120.5 °C (0.000046% wt. by wt.*), Cpd.21 m.p. 80.5-81.5 °C (0.000036% wt. by wt.), Cpd.22 m.p. 147.0-149.0 °C (0.000043% wt. by wt.), Cpd.23 m.p. 260.0-263.0 °C (dec.) (0.00064% wt. by wt.*), Cpd.B and Cpd.C.

The major components in this fraction were Cpd.15, Cpd.17 and Cpd.18.

---

* The percentage yield was calculated compared with dried plant material.

3.10.1 Structural Elucidation of Compound 15

Compound 15 as white amorphous solid, m.p. 85.0-87.0 °C, 2.74 g. (0.0039% wt. by wt. of Fraction VIII), was obtained from the separation of Fraction VIII by silica gel column chromatography in Fraction No. 11-33. This substance did not give only one spot, but it revealed two close spots at Rf 0.32 and 0.29. Although the solvents for recrystallization were changed, this compound still showed two closely spots on TLC plate. Compound 15 gave a deep green colour to Liebermann-Burchard's and also decolourized Br_2 and CCl_4 reagents. This information strongly indicated that it should contain a steroidal skeleton and an unsaturation part (55,60-61).

The IR spectrum of this compound (Fig.139) pointed out the presence of an ester as its functional group at 1740 cm^{-1}. This substance, thus, may be an unsaturated steroidal ester.

The ^1H NMR spectrum (Fig.140) exhibited the olefinic proton signal at 5.38 ppm. (1H,d,J=4.17 Hz). The two signals observed at 4.05 (t,J=6.52 Hz) and 2.27 ppm. (t,J=6.17 Hz) were ascribed for the protons on α-carbon adjacent to ester linkage in alcoholic and acidic parts, respectively. The high intensity signal analogous to methylene groups was present at 1.25 ppm. The signal at 0.88 ppm. should be the methyl proton signal.

The ^{13}C NMR spectrum (Fig.141) gave two carbonyl signals of ester at 173.63 and 173.30 ppm. The two signals which were detected at 139.77 and 122.60 ppm. could be assigned for olefinic carbons. The carbon which attached to oxygen atom of ester was observed at 73.68 ppm. Other signals were corresponded to the signals of methyl, methylene, methine and quarternary carbons. The

high intensity signal at 29.74 ppm. was analogous to several interlinking methylene carbon system. Some parts of the ^{13}C NMR and the ^{13}C NMR off resonance spectra of this compound were resemble to those of β-sitosterol. The comparision of the ^{13}C NMR chemical shifts of this compound with β-sitosterol was given in Table 3.39.

The mass spectrum of this compound (Fig.142) showed a set of molecular ion peaks at m/e (% rel int.) 704.0 (0.14), 676.0 (0.23) and 648.0 (0.20) (Calcd. for $C_{48}H_{96}O_2$, $C_{46}H_{92}O_2$ and $C_{44}H_{88}O_2$: MW. 704.76, 676.72 and 648.69, respectively). The parent ion peaks and the molecular formulae were coincided with saturated long chain aliphatic ester compounds ($CH_3-(CH_2)_m-\overset{O}{\overset{\|}{C}}-O-(CH_2)_n-CH_3$). However, a series of fragmentation ion peaks at m/e 396.0 (28.58), 255.0 (4.53) and 213.0 (3.43), which could not be accounted for the saturated long chain aliphatic ester compounds, was compatible with the fragmentation ion pattern of β-sitosterol. The mass fragmentation pattern of β-sitosterol was presented in Scheme 3.9.

Table 3.39 The comparision of the ^{13}C NMR chemical shifts of Compound 15 and β-sitosterol

Carbon	Chemical shift (ppm.)	
	β-sitosterol	Compound 15
1	37.25	37.06
2	31.66	31.64
3	71.77	71.68
4	45.84	45.88
5	140.72	139.77
6	121.64	122.60
7	32.89	32.88
8	32.89	32.88
9	50.14	50.06
10	36.51	36.62
11	19.39	19.34
12	39.82	39.76
13	45.84	45.88
14	56.75	56.72
15	24.29	24.32
16	39.76	39.76
17	56.07	56.07
18	11.97	12.03
19	19.04	19.07
20	33.95	33.97
21	18.78	18.80
22	36.15	36.19
23	23.07	23.13
24	42.31	42.36
25	28.24	29.25
26	21.10	21.07
27	19.80	19.83
28	26.11	26.17
29	11.86	11.86

The above information, therefore, suggested that this compound should be composed of β-sitosteryl ester whose M^+ was not present and a mixture of three saturated long chain aliphatic ester compounds (waxes).

To prove the presence of β-sitosteryl ester and a mixture of waxes, the basic hydrolysis by 10% KOH in ethanol was set up. The alcoholic parts (Fraction 15A) obtained from the basic hydrolysis reaction revealed two major spots on TLC plate at Rf 0.60 and 0.34 (solvent: chloroform). These two spots were separated by silica gel recolumn chromatography, successively. The first one which was eluted by n-hexane as white amorphous solid. m.p. 74.0-76.0 °C, Rf 0.60 (solvent: chloroform), designated as Compound 15A, gave negative results to both Liebermann-Burchard's and Br_2 in CCl_4 reagents. This result suggested that this fraction should be saturated long chain aliphatic primary alcohols derived from the hydrolysis of waxes.

The IR spectrum of Compound 15A (Fig.143) supported the presence of a saturated long chain primary alcohol. The GLC analysis results (Fig.144) found that this compound contained 6 saturated long chain primary alcohols at Rt 2.40, 3.29, 4.97, 7.60, 12.40 and 15.00 min. which were corresponded to number of carbon 24, 26, 28, 30, 32 and 33, respectively.

Thus, Compound 15A was a mixture of saturated long chain primary alcohols C-24, 26, 28, 30, 32 and 33.

The other part of the alcoholic portion, which was gained from the recolumn chromatography of Fraction 15A by 10% chloroform-hexane in Fraction No. 16-20, was a bright white needle

product, m.p. 134.0-136.0 °C, Rf 0.34 (solvent: chloroform). This compound, labelled as Compound 15B, gave a deep green colour to Liebermann-Burchard's and decolourized Br_2 in CCl_4 reagents which disclosed that the steroidal compound was present in this fraction.

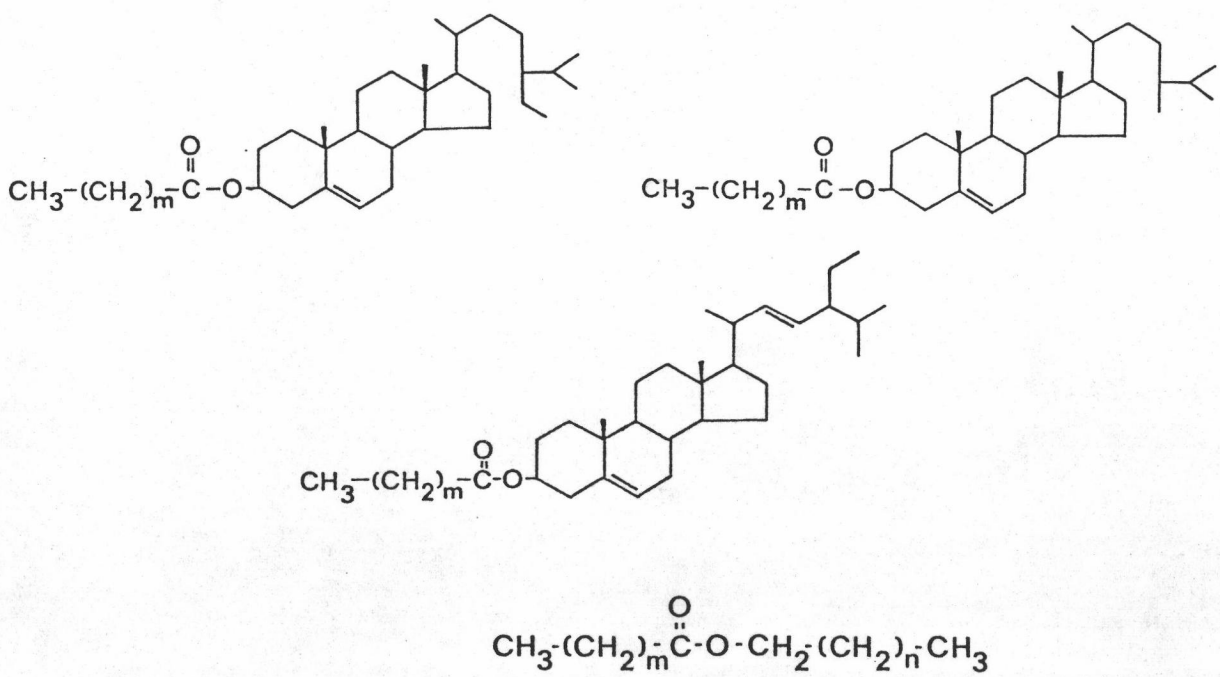
The IR spectrum (Fig.146) revealed the absorption bands coincided with β-sitosterol. The GLC analysis results (Fig.147) revealed one major peak on gas chromatogram at Rt 22.65 min. which was corresponded to the authentic β-sitosterol. Other two minor signals on gas chromatogram were observed at Rt 18.32 and 19.65 min. which were identical to campesterol and stigmasterol, respectively.

Hence, this fraction was steroid compounds containing β-sitosterol as a major component, together with stigmasterol and campesterol as minor ones.

After acidification the acidic portion from the hydrolysis reaction and extraction with diethylether, white amorphous solid, marked as Compound 15C, was obtained. This compound was identified as a saturated long chain aliphatic carboxylic acid by its IR spectrum (Fig.148). The HPLC analysis results (Fig.149) showed that Compound 15C was composed of a mixture of 4 saturated long chain aliphatic carboxylic acids, C-14, 16, 20 and 22, respectively.

In conclusion, supported by colour tests, spectral data and chemical reaction, Compound 15 was consisted of two main types of compounds, i.e., steroidal esters and saturated long chain aliphatic ester compounds (waxes). The steroidal esters did not reveal their molecular ion peaks so that the complete structure could not be elucidated, however, the steroid part in this compound

must be β-sitosterol (as a major component), stigmasterol and campesterol as minor ones. The structure of steroids joined to saturated long chain aliphatic carboxylic acids C-14, 16, 20 or 22 was reasonable for this steroidal ester compound. The waxes in this mixture ought to be at least 3 compounds, i.e, $C_{48}H_{96}O_2$, $C_{46}H_{92}O_2$ and $C_{44}H_{88}O_2$. The possible number of carbon in alcoholic portion was C-24, 26, 28, 32 and 33, while the acidic part should be C-14, 16, 20 and 22. The possible structure of Compound 15 is drawn as shown below:



$$CH_3\text{-}(CH_2)_m\text{-}\overset{O}{\overset{\|}{C}}\text{-}O\text{-}CH_2\text{-}(CH_2)_n\text{-}CH_3$$

m = 14, 16, 20 or 22
n = 24, 26, 28, 32 or 33

Compound 15

3.10.2 Structural Elucidation of Compound 16

Compound 16 was isolated from the silica gel column chromatography of Fraction BB in Fraction No. 6-12. This compound was recrystallized by acetone for several times to yield Compound 16 as white amorphous solid, m.p. 78.0-79.0 °C, 55 mg. (0.08% wt. by wt. of Fraction VIII), Rf 0.34 (solvent: chloroform). This substance showed negative results to all the colour test reagents which revealed that there were neither triterpenoidal or steroidal skeleton, phenolic group, unsaturation part nor carbonyl functional groups in its molecule (55,60-61).

The IR spectrum of this compound (Fig.151) gave the major absorption peaks like those of Compound 6. It showed O-H stretching vibration at 3300 cm^{-1}, C-O stretching vibration of 1 ROH at 1065 cm^{-1} together with other absorption peaks analogous to the vibration modes of methyl and methylene groups (66,67). This information hinted that Compound 16 ought to be a saturated long chain aliphatic primary alcohol.

The ^{1}H NMR spectrum (Fig.152) also supported that this substance should be a long chain compound owing to the high intensity singlet signal at 1.26 ppm. which was corresponded to methylene interlinking system in this compound. Other signals were observed and could be assigned as follows: 3.67 (2H,m, proton on carbinol carbon), 1.52 (2H,s, methylene protons adjacent to carbinol system) and 0.87 (3H,s, methyl protons).

The TLC employing various solvent systems found that this compound gave the same Rf value as those of Compound 6. However, Compound 16 was not acceptable to be Compound 6 because of

their different melting point. (The Compound 6 melted at 83.0-86.0 °C, while the melting point of Compound 16 was 78.0-79.0 °C.). This information hinted that Compound 16 may be composed of a small number of methylene interlinking system in the molecule comparing with Compound 6.

This observation was quite true since both the mass spectrum and the GLC analysis results supported that this compound was the same type as Compound 6, but not identical to. The mass spectrum of Compound 16 (Fig.153) did not give the molecular ion peak, M^+, which was always occurred in a long chain aliphatic primary alcohol compound (68). However, the presence of the dominant fragmentation ion peak corresponded to M^+-H_2O was detected at m/e (% rel.int.) 448.0 (0.67) together with other fragmentation ion peaks at 420.0 (2.90, $448-(CH_2)_2$), 392.0 (11.07, $420-(CH_2)_2$) and other peaks compatible with losing $-(CH_2)_2-$ (m/e 28) step by step. Hence, the possible structure of this compound ought to be dotriacontanol ($C_{32}H_{65}OH$) (MW. 466.52).

Nevertheless, like Compound 6, this compound was infact a mixture of saturated long chain aliphatic primary alcohols by using the analysis of GLC (Fig.154). The results from the GLC analysis revealed that there were 5 substances at Rt 4.89, 6.12, 7.49, 9.40 and 11.62 min. containing in this mixture. From the correlation standard curve between logarithm of retention time and number of standard carbons* (Fig.155), these five compounds were corresponded to carbon 28, 29, 30, 31 and 32, respectively. Besides, from the gas chromatogram cited that the major component in this mixture was at Rt 7.49 min.

It was lucid to conclude that Compound 16 was a mixture of saturated long chain aliphatic primary alcohols; octacosanol ($C_{28}H_{57}OH$), nonacosanol ($C_{29}H_{59}OH$), triacontanol ($C_{30}H_{61}OH$), hentriacontanol ($C_{31}H_{63}OH$) and dotriacontanol ($C_{32}H_{65}OH$). Triacontanol ($C_{30}H_{61}OH$) was the major component in this mixture. The structure of Compound 16 is shown as follow

$$CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}OH \qquad n = 26,27,...,30$$

Compound 16

The composition of saturated long chain aliphatic primary alcohols contained in Compound 16 is presented in Table 3.40.

Table 3.40 The composition of saturated long chain aliphatic primary alcohols contained in Compound 16

Name	Formular	% Composition
octacosanol	$CH_3\text{-}(CH_2)_{26}\text{-}CH_2\text{-}OH$	10.46
nonacosanol	$CH_3\text{-}(CH_2)_{27}\text{-}CH_2\text{-}OH$	1.13
triacontanol	$CH_3\text{-}(CH_2)_{28}\text{-}CH_2\text{-}OH$	72.61
hentriacontanol	$CH_3\text{-}(CH_2)_{29}\text{-}CH_2\text{-}OH$	1.96
dotriacontanol	$CH_3\text{-}(CH_2)_{30}\text{-}CH_2\text{-}OH$	13.84

---

* Standard samples are tetradecanol ($C_{14}H_{29}OH$), hexadecanol ($C_{16}H_{33}OH$), octadecanol ($C_{18}H_{37}OH$), icosanol ($C_{20}H_{41}OH$) and doicosanol ($C_{22}H_{45}OH$).

3.10.3 Structural Elucidation of Compound 17

Compound 17 was isolated from the dichloromethane soluble fraction (Fraction VIII) by silica gel column chromatography in Fraction No. 53-65 by 20% chloroform-hexane. After recrytallization by acetone for several times, Compound 17 as white amorphous solid 1200 mg. (1.71% wt. by wt. of Fraction VIII), m.p. 76.5-78.0 °C, was obtained. This compound gave negative tests to Liebermann-Burchard's, Br_2 in CCl_4, 2,4-DNP and 5% $FeCl_3$ reagents which indicated the absence of triterpenoidal or steroidal skeleton, unsaturation part, carbonyl functional group and phenolic group in its structure (55,60-61).

The IR spectrum of Compound 17 (Fig.156) could be assigned in Table 3.41. From its IR spectrum, this compound revealed a very broad absorption band at 3300-2500 cm^{-1} due to the O-H stretching vibration of carboxylic acid. A strong peak at 1710 cm^{-1} ought to be $-\overset{O}{\overset{\|}{C}}-$ stretching vibration of carboxylic acid and another broad band at 940-930 cm^{-1} was coincided with O-H out-of-plane bending vibration. Other absorption bands were corresponded to methyl and methylene vibration modes (66,67). The information obtained from the IR spectrum suggested that Compound 17 should be a saturated long chain aliphatic carboxylic acid.

Table 3.41 The IR absorption band assignments of Compound 17

Frequency (cm^{-1})	Band type	Tentative assignments
3300-2500	very b	O-H stretching vibration of acid
2920,2850	s	C-H stretching vibration of CH_3-,-CH_2-
1710	s	-C- stretching vibration of acid (O)
1465	m	C-H bending vibration of -CH_2-
1430,1410 1320,1290	m	C-O stretching vibration coupled with with O-H deformation
940-930	b,m	O-H out-of-plane deformation
720	m	C-H rocking mode of -CH_2- (for chain > 4 carbons)

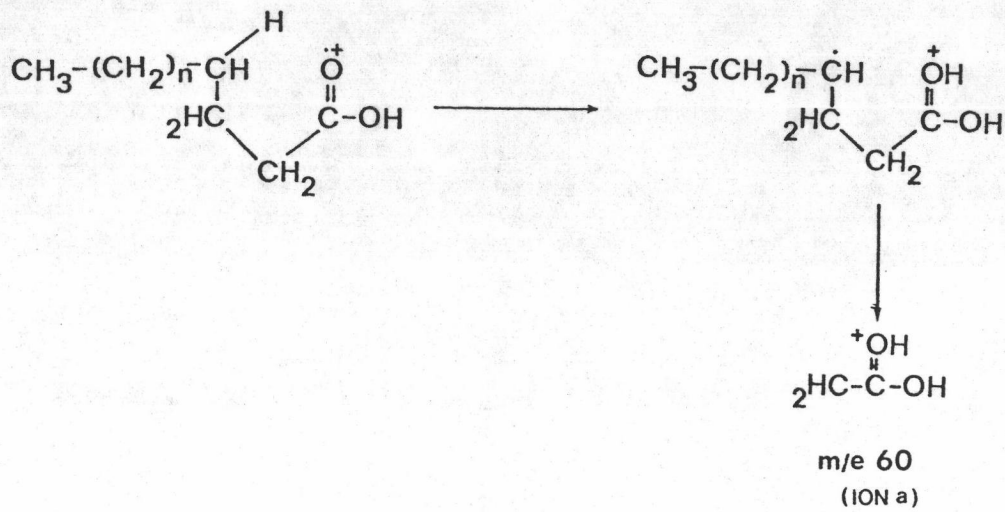
The ^1H NMR spectrum (Fig.157) displayed the proton signals which were supported the IR information that this compound was a long chain substance. It gave a high intensity signal analogous to methylene interlinking system at 1.26 ppm. Other signals were observed at δ (ppm.) 2.35 (2H,t,J=10.52 Hz) corresponded to methylene protons next to carboxyl group (-$\underline{CH_2}$-COOH) and at 0.88 (3H,s) compatible with methyl protons.

The ^{13}C NMR spectrum (Fig.158) displayed the important carbon signal characteristic of carboxyl carbon at 179.75 ppm. Other signals were detected and could be assigned as follows: 14.09 (methyl carbon), 22.70, 24.70, 29.09, 29.25, 29.36, 29.69, 31.96 and 33.97 ppm. corresponded to methylene carbons. The

high intensity signal at 29.69 ppm. was corresponded to the ^1H NMR information which indicated several interlinking methylene groups. The four carbons next to carboxyl group, labelled as α, β, γ, δ, could be tentatively assigned for the signals at 33.97, 31.96, 29.36 and 29.25 ppm., respectively, whereas the three carbons, marked as α′, β′, γ′, adjacent to methyl group were possible to locate at 22.70, 24.70 and 29.09 ppm., respectively.

$$CH_3\overset{\alpha'}{-}CH_2\overset{\beta'}{-}CH_2\overset{\gamma'}{-}CH_2-(CH_2)_n-\overset{\delta}{C}H_2\overset{\gamma}{-}CH_2\overset{\beta}{-}CH_2\overset{\alpha}{-}CH_2-COOH$$

The mass spectrum (Fig.159) gave the molecular ion peak, M^+, at m/e (% rel.int.) 508.0 (0.54) (Calcd. for $C_{34}H_{68}O_2$: MW. 508.54) together with other fragmentation ion peaks at m/e 494.0 (0.62), 480.0 (5.77) and other peaks which were corresponded to be lost $-CH_2-$ (m/e 14) step by step. This fragmentation pattern also supported the existence of long chain saturated compounds (68). Moreover, the presence of the abundant ion peaks at m/e 60.0 (28.85, ion a, which was shown below) and at m/e 73.0 (40.38), 129.0 (63.46) and 185.0 (26.92) (formally $(CH_2)_nCOOH^+$ where n = 2, 6, 10) was found to be the characteristic of a saturated long chain aliphatic carboxylic acid (116).



m/e 60
(ION a)

Supported by the mass spectrum data, this compound was purposed to be tetratriacontanoic acid ($C_{34}H_{68}O_2$). Like compounds 1, 6 and 16, saturated long chain aliphatic compounds always occur in a mixture form. The best way to analyse this type of substance is to use the GLC analysis (69). However, the saturated long chain aliphatic carboxylic acids which contain carbon number more than 20 could not be analysed directly by GLC. Hence, Compound 17 was converted into its methyl ester by using diazomethane reagent and then analysed by GLC. The GLC analysis results (Fig.160) exhibited 11 peaks on gas chromatogram at Rt 3.25, 4.13, 5.26, 6.77, 8.78, 11.33, 14.70, 19.00, 24.63, 32.03 and 41.43 min., respectively. The major one was at 11.33 min. Using the construction of standard correlation curve which was plotted between logarithm of retention time and number of carbons in standard samples*, it was found that the saturated long chain carboxylic acids contained in this mixture was corresponded to number of carbon as 21, 22, 24, 25, 27, 28, 30, 31, 33, 34 and 36, respectively.

According to the spectral evidences and the GLC analysis results, this compound was proved to be a mixture of 11 saturated long chain aliphatic carboxylic acids; henicosanoic acid ($C_{20}H_{41}COOH$), doicosanoic acid ($C_{21}H_{43}COOH$), tetracosanoic acid ($C_{23}H_{47}COOH$), pentacosanoic acid ($C_{24}H_{49}COOH$), heptacosanoic acid ($C_{26}H_{53}COOH$), octasosanoic acid ($C_{27}H_{55}COOH$), triacontanoic acid

---

* Standard samples are the methyl ester of dodecanoic acid ($C_{11}H_{23}COOH$), tetradecanoic acid ($C_{13}H_{27}COOH$), hexadecanoic acid ($C_{15}H_{31}COOH$) and octadecanoic acid ($C_{17}H_{35}COOH$).

($C_{29}H_{59}COOH$), hentriacontanoic acid ($C_{30}H_{61}COOH$), tritriacontanoic acid ($C_{32}H_{65}COOH$), tetratriacontanoic acid ($C_{33}H_{67}COOH$) and hexatriacontanoic acid ($C_{35}H_{71}COOH$). The major component in this compound was octacosanoic acid ($C_{27}H_{55}COOH$). The structure of Compound 17 is shown below:

$$CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}COOH \qquad n = 18,19,21,22,24,25,27,28,30,31,33$$

Compound 17

The composition of saturated long chain aliphatic carboxylic acids contained in Compound 17 is shown in Table 3.42.

Table 3.42 The composition of saturated long chain aliphatic carboxylic acids contained in Compound 17

Name	Formular	% Composition
henicosanoic acid	$CH_3\text{-}(CH_2)_{18}\text{-}CH_2\text{-}COOH$	0.16
doicosanoic acid	$CH_3\text{-}(CH_2)_{19}\text{-}CH_2\text{-}COOH$	0.29
tetracosanoic acid	$CH_3\text{-}(CH_2)_{21}\text{-}CH_2\text{-}COOH$	3.86
pentacosanoic acid	$CH_3\text{-}(CH_2)_{22}\text{-}CH_2\text{-}COOH$	9.48
heptacosanoic acid	$CH_3\text{-}(CH_2)_{24}\text{-}CH_2\text{-}COOH$	23.43
octacosanoic acid	$CH_3\text{-}(CH_2)_{25}\text{-}CH_2\text{-}COOH$	37.76
triacontanoic acid	$CH_3\text{-}(CH_2)_{27}\text{-}CH_2\text{-}COOH$	12.43
hentriacontanoic acid	$CH_3\text{-}(CH_2)_{28}\text{-}CH_2\text{-}COOH$	3.48
tritriacontanoic acid	$CH_3\text{-}(CH_2)_{30}\text{-}CH_2\text{-}COOH$	5.78
tetratriacontanoic acid	$CH_3\text{-}(CH_2)_{31}\text{-}CH_2\text{-}COOH$	1.87
hexatriacontanoic acid	$CH_3\text{-}(CH_2)_{33}\text{-}CH_2\text{-}COOH$	1.45

3.10.4 Structural Elucidation of Compound 18

Compound 18, one of the major components of Fraction VIII, was separated from the heartwoods of R. apiculata by silica gel column chromatography in Fraction No. 66-76. After recrystallization with a mixture of dichloromethane and n-hexane, Compound 18 as bright white needle, 870 mg. (1.24% wt. by wt. of Fraction VIII), Rf 0.32 (solvent: chloroform), m.p. 139.0-141.0 °C was obtained. This compound gave a deep green colour to Liebermann-Burchard's and also decolourized Br_2 in CCl_4 reagents. This information was indicative of the unsaturated steroidal structure (55,60-61).

The IR spectrum (Fig.162) of Compound 18 was coincided with that of Compound 10. It showed the hydroxy absorption band at 3430 cm^{-1}. The weak and broad absorption band at 1670-1630 cm^{-1} might be due to the C=C stretching vibration of trisubstituted olefin structure (-C=CH-). The prominent band at 1050 cm^{-1} agreed with the C-O stretching vibration of an equatorial hydroxy group, while the two bands at 840 and 800 cm^{-1} which were C-H out-of-plane bending were compatible to the characteristic absorption bands of Δ^5-3-β-hydroxy steroids.

The mass spectrum (Fig.163) gave the same molecular ion peaks as Compound 10, i.e., it gave the parent ion peaks and the fragmentation ion pattern identical to β-sitosterol (M^+ 414.0), stigmasterol (M^+ 412.0) and campesterol (M^+ 400.0). Other fragmentation ion pattern was also superimposed to these three steroids.

Therefore, one of the major components of Fraction VIII was a mixture of β-sitosterol, stigmasterol and campesterol.

However, the melting point of this compound was not equal to that of Compound 10, it gave the melting range near the melting point of the reported β-sitosterol (Lit.(74) 134 °C) rather than Compound 10 did. Hence, this mixture may be composed of β-sitosterol in the higher ratio than it contained in Compound 10.

The GLC analysis results (Fig.164) gave good agreement to this observation, i.e, this mixture contained three steroids β-sitosterol, stigmasterol and campesterol, but not the same ratio as in Compound 10. The composition of these three steroids in Compound 18 is shown in Table 3.43.

Table 3.43 The composition of steroids contained in Compound 18

Name	Rt (min.)	% Composition
campesterol	18.28	4.61
stigmasterol	19.58	18.47
β-sitosterol	22.41	76.92

3.10.5 Structural Elucidation of Compound 19

Compound 19 was obtained from the combination of Fraction 121-132. After recrystallization with a mixture of dichloromethane and n-hexane for several times, bright yellow needle sublimated at 230 °C, 380 mg. (0.59% wt. by wt. of Fraction VIII), was derived. This compound showed only one spot on TLC plate at Rf

value 0.56 using 5% methanol in chloroform as a mobile phase. It showed negative tests to Liebermann-Burchard's and 5% $FeCl_3$ reagents which indicated the absence of steroids, triterpenoids and phenolic compounds, respectively. However, the colour tests of this substance suggested the presence of carbonyl and unsaturation moiety as its functional groups, since it gave a positive test to 2,4-DNP and decolourized Br_2 in CCl_4 reagents, respectively (55,60-61).

The IR spectrum (Fig.165) which is assigned in Table 3.44, revealed the most notable stretching vibration bands at 1695, 1650 and 1625 due to $-\overset{O}{\overset{\|}{C}}-$ stretching vibration in conjugated systems (66,67). The absorption bands belonged to unsaturation moiety were observed at 3060 (C-H stretching vibration of $R_1R_2C{=}CR_3H$), 1595 (C=C) and the sharp peak at 880 (C-H out-of-plane deformation of $R_1R_2C{=}CR_3H$). The peaks at 1260, 1220 and 1105 were consistent with the absorption of C-O stretching vibration of ether.

This compound was purposed the structural formular to be $C_8H_8O_4$ according to the mass spectrum data and the elemental analysis result. From its formular implied the high degree of unsaturation, i.e., ring + double bond = 5.

The UV spectrum (Fig.169) gave the maxima absorption peaks at 287 and 376 nm. ($\log \varepsilon$ = 4.34 and 3.01, respectively) which pointed out the presence of α , β-unsaturated ketone (71).

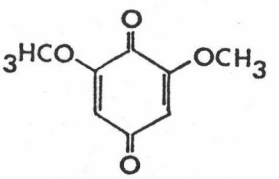
Table 3.44 The IR absorption band assignments of Compound 19

Frequency (cm^{-1})	Band type	Tentative assignments
3060	w	C-H stretching vibration of $R_1R_2C=CR_3H$
2950	w	C-H stretching vibration of CH_3-
1695,1650 1625	s	-C- stretching vibration of unsaturated ketone
1595	s	C=C stretching vibration
1450,1380	w	C-H bending vibration of CH_3-
1320	s	O-C-C stretching vibration coupled with bending vibration of C-C-C
1260,1220	s	C-O asymmetric stretching vibration of ether
1105	s	C-O symmetric stretching vibration of ether
880	m	C-H out-of-plane deformation of $R_1R_2C=CR_3H$

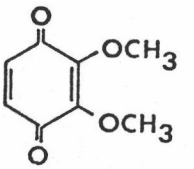
The above information hinted that this compound should be a member of quinone compounds with containing methoxy groups as its substituents. However, there are two possible types of quinones, i.e., o- and p-quinones (135). The best data which could be used to distinguish these two kinds is the maxima absorptions due to n- π^* and π - π^* in the UV and the visible spectrum. The o-quinones always give the λ max belonging to n- π^* in range of 500-650 nm. which is higher than p-isomers (300-500 nm.)

(135-136). The result from the UV spectrum of this compound gave the maximum absorption peak at 287 nm. possibly due to $\pi - \pi^*$, while the λ max at 376 nm. was the characteristic of $n-\pi^*$ of p-quinonoid structure. This information, hence, was obvious to show that Compound 19 contained p-quinonoid structure.

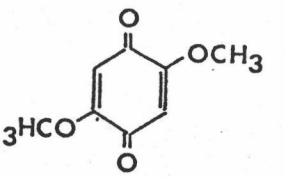
The three possible structures of Compound 19, thus, could be drawn as follows:



(I)



(II)



(III)

The ^1H NMR spectrum (Fig.167) exhibited the signals corresponded to the structures(I) and (III). Since it showed two singlet signals at 5.86 ppm. (2H) compatible with olefinic protons and at 3.83 ppm. (6H) attributed to methoxy protons.

The ^{13}C NMR spectrum (Fig.168) showed the 5 carbon signals at 186.85 and 176.72 ppm. which were ascribed for the carbonyl carbons of quinonoid skeleton. The signals at 157.32 and 107.43 (2C each) were consistent with the olefinic carbons and the signal at 56.20 (2C) was coincided with methoxy carbons.

The ^1H NMR and the ^{13}C NMR spectra implied that this quinone should have a symmetrical structure. Hence, it was lucid to state that the structure (I) ought to be the appropriate one for Compound 19. (The ^{13}C NMR spectrum of the structure (III) should give only four carbon signals, since the two carbonyl carbons were

around the same environment). The ^{1}H NMR and the ^{13}C NMR chemical shift assignments of Compound 19 are shown below:

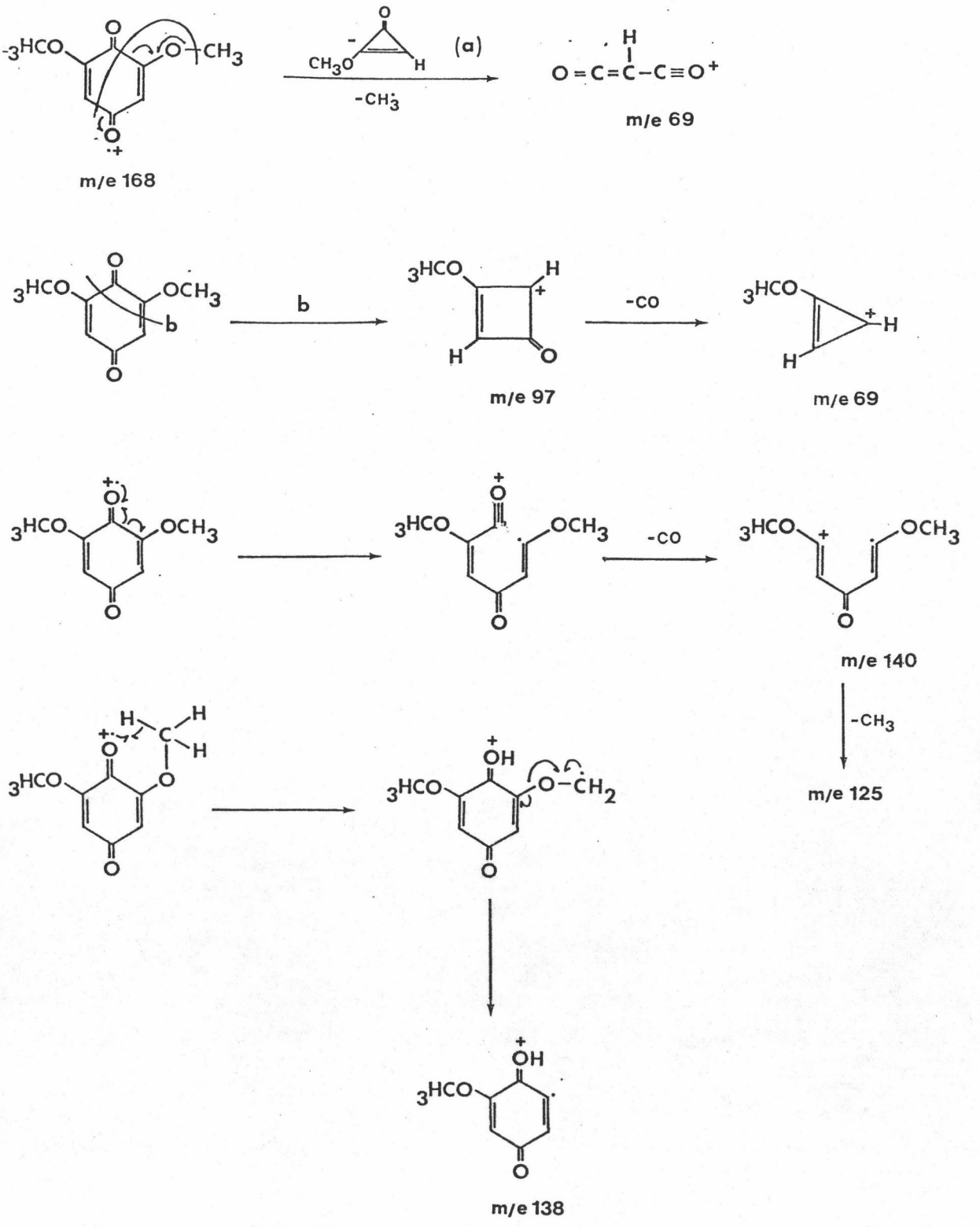
Table 3.45 The ^{1}H NMR and the ^{13}C NMR chemical shift assignments of Compound 19

Position	Chemical shift (ppm.)	
	^{1}H NMR	^{13}C NMR*
1	-	186.85 (s)
2,6	-	157.32 (s)
3,5	5.86	107.43 (d)
4	-	176.72 (s)
$-OCH_3$	3.83	56.50 (q)

* The letter in the blanket is the multiplicity obtained from the ^{13}C NMR off resonance spectrum.

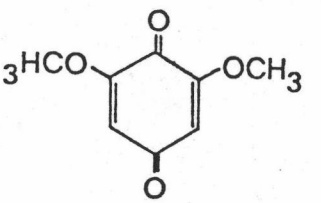
The mass spectrum (Fig.166) was also supported the structure (I). It gave the parent ion peak at m/e (% rel. int.) 168.0 (100.00) (Calcd. for $C_8H_8O_4$: MW. 168.06) together with other fragmentation ion peaks at 153.0 (81.00), 140.0 (9.06), 138.0 (28.94), 125.0 (14.10), 97.0 (11.04) and 69.0 (85.35). The possible mass fragmentation pattern is given in Scheme 3.19 (116).

Scheme 3.19 The possible mass fragmentation pattern of Compound 19



To confirm this structure, 2,6-dimethoxy-p-benzoquinone was synthesized from 1,3,5-trimethoxybenzene (65). The comparision by both physical properties and spectral data between the synthetic 2,6-dimethoxy-p-benzoquinone and the isolated one was found that these two substances were identical to each other in all respects.

According to the spectral evidences, colour tests and comparision with the synthetic one, Compound 19 was no doubt to be concluded to be 2,6-dimethoxy-p-benzoquinone. The structure of this compound is shown below:



Compound 19

2,6-Dimethoxy-p-benzoquinone

3.10.6 Structural Elucidation of Compound 20

Compound 20 was separated from recolumn chromatography of Fraction FF1 by using 30% chloroform-hexane as an eluent. After purification by recrystallization with hot n-hexane for five times, Compound 20 as white needle crystal, 32 mg. (0.04% wt. by wt. of Fraction VIII)., m.p. 109.0-110.5 °C, was obtained. This compound showed only one spot on TLC plate at Rf 0.48 (solvent: 5% methanol-chloroform). Compound 20 gave a negative test to Liebermann-Burchard's and could not decolourize Br_2 in CCl_4 reagents, but it revealed a deep green colour to 5% $FeCl_3$ and gave a

positive test to 2,4-DNP reagents. The colour test implied that this compound was not an unsaturated steroidal or triterpenoidal compounds, but Compound 20 should be a phenolic compound containing a carbonyl functional group.

The IR spectrum (Fig.173), assigned in Table 3.46, revealed the broad absorption bands at 3290 cm^{-1} which was corresponded to O-H stretching vibration. The strong peak at 1670 cm^{-1} could be assigned for $-\overset{O}{\overset{\|}{C}}-$ stretching vibration of conjugated carbonyl group. The most notable C-O stretching vibrations could be ascribed as follows: 1250, 1135 and 1100 (C-O stretching vibration of ether), 1205 (C-O stretching vibration of phenol). The absorption peaks due to C-H out-of-plane deformation of aromatic nucleus were observed at 840 and 830 cm^{-1}.

Supported by the mass spectrum data and the elemental analysis result, the molecular formular of this compound was proposed to be $C_9H_{10}O_4$ (MW.: 182.08). The proposed formular implied the high degree of unsaturation, i.e., ring + db. = 5.

The UV spectrum (Fig.177) gave the maxima absorption peaks (λ max) at 227 nm. (log ε = 4.60) and 309 nm. (log ε = 4.53) which suggested the presence of a substituted benzene ring (67).

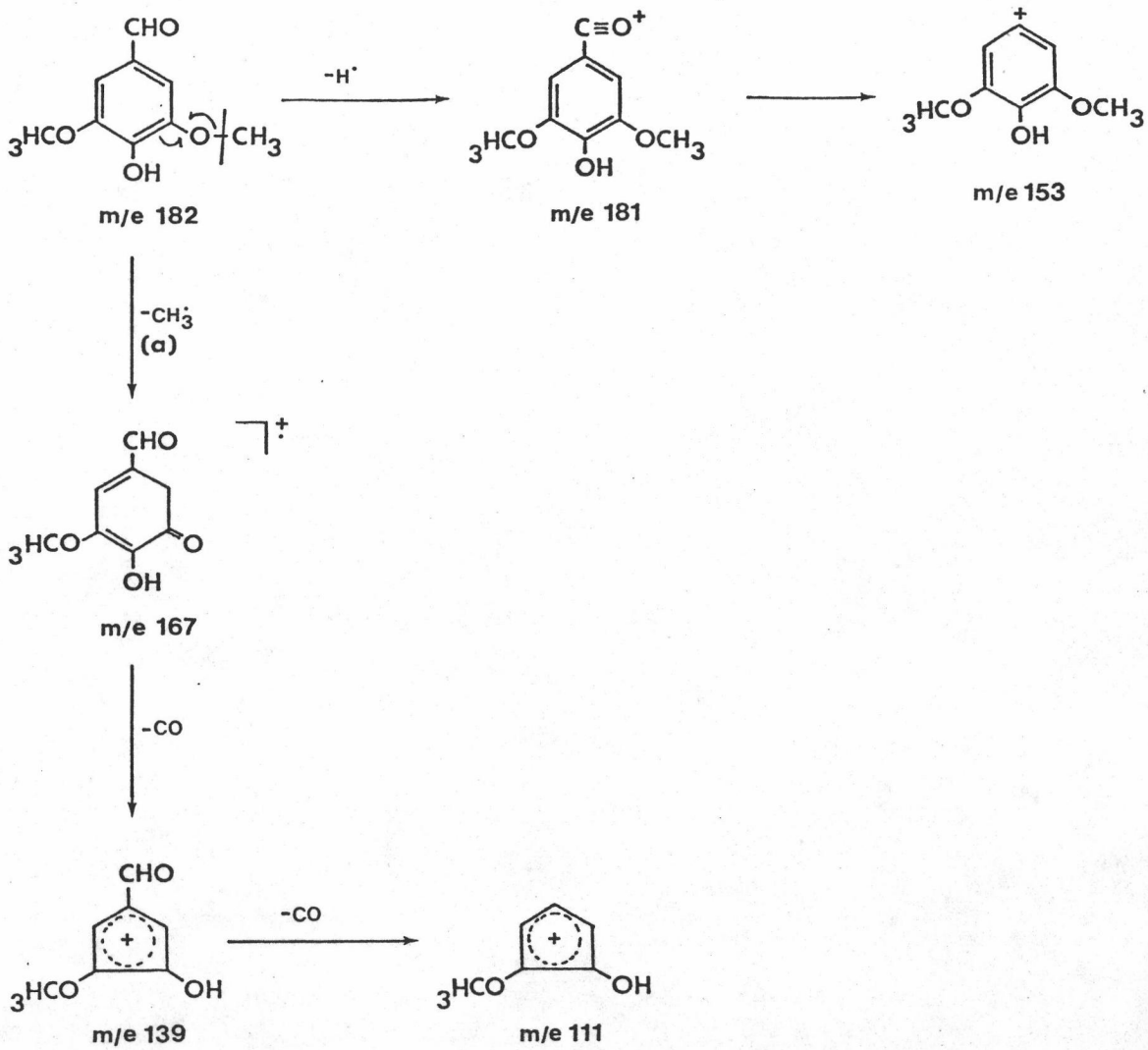
Table 3.46 The IR absorption band assignments of Compound 20

Frequency (cm^{-1})	Band type	Tentative assignments
3290	b	O-H stretching vibration
3050	w	C-H stretching vibration of alkene or aromatic
2980,2950	w	C-H stretching vibration of CH_3-
2850,2870	w	C-H stretching vibration of aldehyde
1670	s	-C(=O)- stretching vibration of conjugated carbonyl system
1605,1595 1510,1475 1450	s	C=C stretching vibration of alkene or aromatic
1320	s	C-C(=O)-H stretching vibration couple with C-C-H bending vibration
1250	s	C-O stretching vibration of dialkyl ether
1205	s	C-O stretching vibrration couple with O-H deformation of phenol
1135,1100	s	C-O symmetrical stretching vibration of dialkyl ether
905	w	C-H deformation of aldehyde
840,830	m	C-H out-of-plane deformation of aromatic nucleus

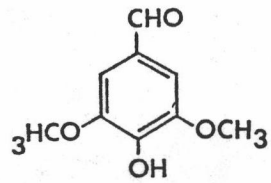
The ^{1}H NMR spectrum (Fig.175) showed the singlet signal at 9.82 ppm. with 1H integration which was ascribed for an aldehyde proton. Two proton singlet signal at 7.15 ppm. was the characteristic of aromatic protons. The broad signal at 6.10 ppm. with intensity corresponding to 1H, disappeared when being shaked with D_2O, was compatible with a phenolic proton, and the sharp singlet at 3.97 ppm. for six protons were attributed to methoxy protons. The singlet signal belonged to aromatic protons suggested that Compound 20 ought to be a symmetrical structure.

The mass spectrum of this compound (Fig.174) gave the molecular ion peak and base peak at m/e (% rel int.) 182.0 (100.00, M^+) (Calcd. for $C_9H_{10}O_4$: MW. 182.08). The fragmentation peaks at m/e 181.0 (52.62) was due to the loss of 1H from the molecular ion peak, this information was corresponded to the presence of aldehyde functional group in its structure (68). Other fragmentation peaks were observed at m/e 167.0 (15.76, M^+-CH_3), 153.0 (6.15, M^+-CHO), 139.0 (13.92) and 111.0 (14.97). The possible mass fragmentation pattern is presented in Scheme 3.20.

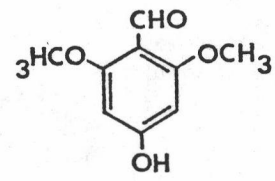
Scheme 3.20 The possible mass fragmentation ion pattern of Compound 20



From the above spectral information, the possible structure of this compound was an aromatic aldehyde which contained hydroxy and methoxy groups as its substituents. Two possible structure could be drawn as follows:



(I)



(II)

The ^{13}C NMR (Fig.176) and the ^{13}C NMR off resonance spectra (Fig.176b) exhibited the chemical shift of carbon corresponded to an aldehyde carbon at 190.75 ppm. The aromatic carbons were observed at 163.99, 147.30, 128.40 and 106.78 ppm. The chemical shift at 76.45 ppm.(2C) was consistent with methoxy carbons.

The calculation for incremental shifts of aromatic carbon atoms of benzene could be used to distinguish those two possible structures (71). The calculation data was given in Table 3.47. The ^{1}H NMR and the ^{13}C NMR chemical shift assignments are presented in Table 3.48.

Table 3.47 The calculation of incremental shifts of aromatic carbon atoms of Compound 20

Structure	Substituents	Aromatic carbons					
		C-1	C-2	C-3	C-4	C-5	C-6
I		128.5	128.5	128.5	128.5	128.5	128.5
	$-\overset{O}{\overset{\|}{C}}-H$	+8.6	+1.3	+0.6	+5.5	+0.6	+1.3
	$-OCH_3$ (3)	+1.0	-14.4	+31.4	-14.4	+1.0	-7.7
	$-OCH_3$ (5)	+1.0	-7.7	+1.0	-14.4	+31.4	-14.4
	-OH	-7.3	+1.4	-12.7	+26.9	-12.7	+1.4
		131.8	109.1	148.8	132.1	148.8	109.1
II		128.5	128.5	128.5	128.5	128.5	128.5
	$-\overset{O}{\overset{\|}{C}}-H$	+8.6	+1.3	+0.6	+5.5	+0.6	+1.3
	$-OCH_3$ (2)	-14.4	+31.4	-14.4	+1.0	-7.7	+1.0
	$-OCH_3$ (6)	-14.4	+1.0	-7.7	+1.0	-14.4	+31.4
	-OH	-7.3	+1.4	-12.7	+26.9	-12.7	+1.4
		101.0	163.6	94.3	162.9	94.3	163.6

Note The observed signals were presented at δ (ppm.): 190.75 ($-\underline{\overset{O}{\overset{\|}{C}}}-H$), 163.99, 147.30, 128.40 and 106.78 (aromatic carbons) and 56.45 (methoxy carbons).

Table 3.48 The ^{1}H NMR and the ^{13}C NMR chemical shift assignments of Compound 20

Position	Chemical shifts (ppm.)	
	^{1}H NMR	^{13}C NMR*
1	-	128.40 (s)
2,6	7.15	106.78 (d)
3,5	-	147.30 (s)
4	-	163.99 (s)
$-\overset{O}{\overset{\|}{C}}-H$	9.42	190.75 (s)
-OH	6.10	-
$-OCH_3$	3.97	56.45 (q)

* The letter in the blanket is the multiplicity obtained from the ^{13}C NMR off resonance spectrum.

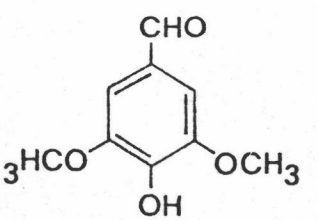
From the above data including the incremental shift calculations, the structure (I) was found to be corresponded to Compound 20 rather than the structure (II).

Literature searches on these two compounds found that the 3,5-dimethoxy-4-hydroxy benzaldehyde (I) and 2,6-dimethoxy-4-hydroxy benzaldehyde (II) revealed significantly different melting point. Thus, this physical property could be used to confirm that Compound 20 should be either 3,5-dimethoxy-4-hydroxy benzaldehyde (syringaldehyde) or 2,6-dimethoxy-4-hydroxy benzaldehyde. The comparision data between these two compounds is given in Table 3.49.

Table 3.49 The comparision data of 3,5-dimethoxy-4-hydroxybenzaldehyde (syringaldehyde) and 2,6-dimethoxy-4-hydroxybenzaldehyde

Compounds	melting point (°C)	Ref.
syringaldehyde	113.0	137
2,6-dimethoxy-4-hydroxybenzaldehyde	234.0	138
Compound 20	109.0-110.5	-

Supported by colour tests and spectroscopic data, Compound 20 was obvious to conclude its structure to be syringaldehyde; the structure is shown below:



Compound 20
Syringaldehyde

3.10.7 Structural Elucidation of Compound 21

Compound 21 was collected from the silica gel column chromatography of Fraction VIII in Fraction No. 166-178 by 80% chloroform-hexane. This compound was purified by recrystallization with a mixture of acetone and methanol twice and then hot n-hexane twice to yield Compound 21 as white amorphous product, m.p. 80.5-

81.5 °C, 20 mg. (0.03% wt. by wt. of Fraction VIII). This substance revealed only one spot on TLC plate at Rf 0.75 using 20% methanol-chloroform as a developing solvent. Compound 21 gave negative tests to all colour test reagents which suggested the absence of a triterpenoidal or steroidal skeleton, unsaturation part, carbonyl functional group and phenolic group in its structure.

The IR spectrum (Fig.178) which was assigned in Table 3.50, showed the absorption bands at 3420 and 1020 cm^{-1} which were the characteristic of O-H and C-O stretching vibration of primary alcohol. The peaks at 1740 and 1170 cm^{-1} were attributed to the $-\overset{O}{\overset{\|}{C}}-$ and C-O stretching vibration of ester, respectively. Other peaks in this spectrum were coincided with a saturated long chain aliphatic hydrocarbon. From the IR spectrum information, the possible structure for Compound 21 was a long chain ester containing a hydroxyl group (primary alcohol) as its substituent.

The ^{1}H NMR spectrum (Fig.180) showed the proton signal at 4.15 ppm. which was assigned for the α-proton in an alcoholic portion of ester molecule ($R-\overset{O}{\overset{\|}{C}}-O-\underset{H}{\overset{|}{C}}-R'$), while the triplet signal at 2.35 ppm. was the α-proton in an acidic portion ($R-\underset{H}{\overset{|}{C}}-\overset{O}{\overset{\|}{C}}-O-R'$). The signal at 3.93 ppm. ought to be the proton on carbon which was adjacent to a hydroxyl group. Another high intensity and sharp singlet peak at 1.26 ppm. was consistent with several interlinking methylene protons in the molecule of this compound. The rest two signals at 1.56 and 0.88 ppm. were corresponded to methylene protons and methyl protons, respectively.

Table 3.50 The IR absorption band assignments of Compound 21

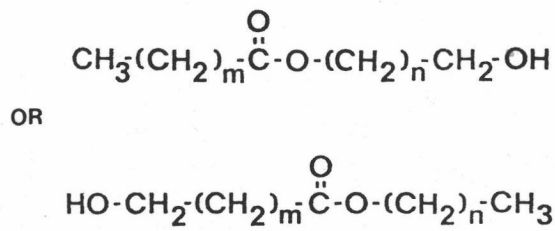
Frequency (cm^{-1})	Band type	Tentative assignments
3420	b	O-H stretching vibration
2920,2850	s	C-H stretching vibration of CH_3-, -CH_2-
1740	m	-C- stretching vibration of ester (O)
1465	m	C-H bending vibration of -CH_2-
1170	m	C-O stretching vibration of ester
1020	w	C-O stretching vibration of 1 ROH
730,720	w	C-H rocking vibration mode for carbon chain > 4

Owing to a small amount of sample, the ^{13}C NMR spectrum (Fig.181) did not give the complete information, i.e., the absence of a carbonyl peak of ester. However, the presence of the carbon signal at 29.69 ppm. which was analogous to methylene protons was observed. The two carbon signals at 65.12 and 63.11 ppm. may be due to the carbons attached to oxygen atom of ester and alcohol, respectively. Other signals around 39.87 to 14.03 ppm. were compatible with methylene and methyl carbon signals.

The possible structural formular of this compound was proposed on the basis of the mass spectrum result. From the mass spectrum, it gave four sets of signal at m/e (% rel int.) 846.0 (0.98), 818.0 (0.98), 790.0 (0.73) and 762.0 (0.19) which were corresponded to $C_{57}H_{114}O_3$, $C_{55}H_{110}O_3$, $C_{53}H_{106}O_3$ and $C_{51}H_{102}O_3$: MW.

846.90, 818.87, 790.83 and 762.80 ,respectively). The fragmentation pattern of this compound was similar to that of a long chain compound.

Basing on the spectral evidences, the possible structure of Compound 21 could be drawn as follows:



OR

m+n = 48,50,52 and 54

Compound 21

3.10.8 Structural Elucidation of Compound 22

Compound 22 was isolated from a dichloromethane soluble fraction (Fraction VIII) by silica gel column chromatography in Fraction No. 207-217 using 5% methanol in chloroform as an eluent. After purification by recrystallization with a mixture of chloroform and methanol for three times, white amorphous solid, m.p. 145.0-147.0 °C, Rf 0.60 (solvent: 15% methanol-chloroform), was obtained. Compound 22 gave negative tests to all colour test reagents which suggested the absence of steroids, triterpenoids, unsaturation parts, phenolic compounds and carbonyl functional group in its structure.

The IR spectrum (Fig.182) which is assigned as given in Table 3.51, exhibited the important signals characteristic of primary amide at 3350, 3200 (d,N-H asym. and sym. stretching vibration) and 1620 (-C- stretching vibration of amide). Other absorption bands were observed at 2900, 2850, .1475 and 720

corresponded to a long chain aliphatic compound.

Table 3.51 The IR absorption band assignments of Compound 21

Frequency (cm^{-1})	Band type	Tentative assignments
3350,3200	d	N-H asymmetrical and symmetrical stretching vibration
2900,2850	s	C-H stretching vibration of CH_3-, $-CH_2-$
1620	m	$-\overset{O}{\overset{\|}{C}}-$ stretching vibration of amide
1520	m	N-H in-plane bending vibration
1475,1460	m	C-H bending vibration of CH_3-, $-CH_2-$ and C-N stretching vibration coupled with N-H deformation
720	w,b	N-H out-of-plane deformation or C-H rocking mode for carbon chain > 4

The ^1H NMR spectrum (Fig.183) displayed the high intensity proton signal belonging to methylene interlinking system at 1.25 ppm. and another signal at 0.87 ppm. due to the proton signal of methyl group.

From the above information, Compound 21 was proposed to be a saturated long chain aliphatic primary amide.

The mass spectrum (Fig.184) gave a set of molecular ion peaks at m/e (% rel.int.) 703.0 (0.41), 689.8 (1.26), 675.0 (6.83), 661.0 (10.41) and 647.0 (13.77) (Calcd. for $C_{48}H_{97}ON$, $C_{47}H_{95}ON$, $C_{46}H_{93}ON$, $C_{45}H_{91}ON$ and $C_{44}H_{89}ON$: MW. 703.77, 689.76,

675.74, 601.72 and 647.71, respectively). The mass fragmentation pattern of this compound supported that this compound ought to be a long chain aliphatic substance.

According to spectroscopic results, this compound could be concluded the structure to be a mixture of tetratetracontanamide ($C_{44}H_{89}ON$), pentatetracontanamide ($C_{45}H_{91}ON$), hexatetracontanamide ($C_{46}H_{93}ON$), heptatetracontanamide ($C_{47}H_{95}ON$) and octatetraconatamide ($C_{48}H_{97}ON$). The structure of this compound was shown below:

$$CH_3(CH_2)_n\text{-}\overset{\overset{O}{\|}}{C}\text{-}NH_2 \qquad n = 41,42,\ldots,45$$

Compound 22

3.10.9 Structural Elucidation of Compound 23

Compound 23 was isolated from both silica gel column chromatography of Fraction VIII in Fraction No. 218-227 and aluminium oxide column chromatography of Fraction XI in Fraction No. 53-62 by 20% methanol-chloroform. After purification by recrystallization this compound with hot ethanol, a white amorphous product 450 mg., m.p. 260.0-263.0 °C (dec.), Rf 0.89 (solvent: 20% methanol-chloroform) was obtained. The colour tests of this compound revealed the possibility of an unsaturated steroidal glycoside, since it showed a deep green colour to Liebermann-Burchard's, decolourized Br_2 in CCl_4 and gave a positive test to Molisch's reagents.

The IR spectrum of this compound (Fig.185) showed the absorption bands at 3420 cm^{-1} which was due to O-H stretching vibration, 1640 cm^{-1} attributed to C=C stretching vibration, 1080-

1020 cm^{-1} concided with C-O stretching vibration of glycosidic linkage and 890 cm^{-1} compatible with the anomeric axial C-H deformation. The IR spectrum of this compound was found to be similar to that of Compound 14.

The Co. TLC in several developing solvent systems between Compound 23 and Compound 14 was carried out and found that these two compounds gave the same Rf values in various solvent systems. The mixed m.p. of both compounds did not show any depression.

By direct comparision with the authentic sample, Compound 23 was obvious to be concluded to be β-sitosteryl-3-O- β - D-glucopyranoside.

3.11 Chemical Constituents of Fraction IX

Fraction IX, as a white solid 14.00 g. (0.02% wt. by wt. of plant material), was obtained during evaporating the solvent from 95% ethanol soluble fraction. Its physical properties and ignition test hinted that this fraction was inorganic salts. The qualitative analysis found that Fraction IX was mainly composed of chloride salts of sodium and potassium. The FES analysis results for determination the quantities of cations revealed that this fraction was consisted of sodium as a major component 99.98% together with potassium and magnesium as trace elements.

3.12 Chemical Constituents of Fraction X

Fraction X was a brown-reddish crude 321.00 g. (0.46% wt. by wt. of plant material) which was insoluble in both chloroform and

water. The screening tests of this fraction were carried out in the similar way to Fraction III and found that Fraction X was a condensed and hydrolysable tannin fraction.

3.13 Chemical Constituents of Fraction XI

Fraction XI was a chloroform soluble fraction of the heartwoods of R. apiculata, 195.00 g. (0.28% wt. by wt. of plant material). The separation of this fraction by aluminium oxide column chromatography yielded only one crystalline product 300 mg. (0.46% wt. by wt. of Fraction XI). After purification by recrystallization with hot ethanol, white amorphous product, m.p. 260.0-263.0 °C (dec.), Rf 0.89 (solvent: 20% methanol-chloroform), was obtained. This substance was found to be identical to Compound 23 by direct comparisions such as mixed m.p., Co. TLC and comparision of their IR spectra. Therefore, the major component in this fraction was β-sitosteryl-3-O-β-D-glucopyranoside.

3.14 Chemical Constituents of Fraction XII

Fraction XII was a n-butanolic soluble fraction 214.70 g. (0.31% wt. by wt. of plant material) which was gained by the extraction of the aqueous layer with n-butanol. This fraction was preliminary examined like Fraction V (the butanolic extract of the leaves of R. apiculata). The colour test results obtained from these two parts were different, i.e, Fraction V revealed a positive test for flavone compounds (Shinoda's reagent), while this fraction did not. However, Fraction XII showed a deep blue colour to 5% $FeCl_3$ and gave a positive test to Molisch's reagents like Fraction IV.

Thus, this fraction ought to contain phenolic and carbohydrate compounds. The further extraction procedure for this fraction 20.00 g. was carried out in the same way as Fraction V to gain an ethylacetate soluble fraction 5.39 g. (26.95% wt. by wt. of Fraction XII) and a water soluble fraction 7.45 g. (37.25% wt. by wt. of Fraction XII), respectively. The ethylacetate fraction displayed the possibility to contain phenolic compounds, since it still gave a deep blue colour to 5% $FeCl_3$, whereas the water soluble fraction exhibited the presence of carbohydrates.

Like Fraction V, the ethylacetate fraction was further examined on phenolic compounds and gibberellins by HPLC analysis.

The HPLC analysis results for phenolic compounds stated that this fraction should contain gallic acid, catechol and p-hydroxybenzoic acid and may be consisted of resorcinol, m-hydroxybenzoic acid, tannic acid, benzoic acid and salicylic acid. The results of this investigation are tabulated as shown below:

Table 3.52 The HPLC analysis results for phenolic compounds in Fraction XIIA

Rt (min.)	Indication	Rt (min.)	Indication
3.36	gallic acid	12.89	m-hydroxybenzoic acid
5.91	(resocinol)		
6.72	catechol	20.26	
9.69	p-hydroxybenzoic acid	21.06	tannic acid
		22.72	benzoic acid
11.37		26.12	salicylic acid
		29.22	

The HPLC analysis results for gibberellins gave the same result as that of the leaves, i.e., it revealed the possibility to contain gibberellin A_3.

3.15 Chemical Constituents of Fraction XIII

Fraction XIII was a water soluble part 366.50 g. (0.52% wt. by wt. of plant material). This fraction was further extracted by methanol at room temperature to gain a methanol soluble fraction (Fraction XIIIA, 36.10% wt. by wt of fraction XIII) and a methanol insoluble fraction (Fraction XIIIB, 50.86% wt. by wt. of Fraction XIII).

Study on Fraction XIIIA

The preliminary colour tests of this fraction displayed that Fraction XIIIA contained carbohydrate (reducing sugar) and amino acids. The investigation on carbohydrate was performed in the similar way to Fraction VIA. The results from various methods were coincided that the major sugar component in Fraction XIIIA was arabinose. The examination on amino acids in this fraction was performed by Amino Acid Analyser. The results of qualitative and quantitative analysis of amino acids in Fraction XIIIA are shown in Table 3.53 (see also Fig.190).

Table 3.53 The qualitative and quantitative analysis results for amino acids in Fraction XIIIA

Amino acids	Concn*	Amino acids	Concn*
aspartic	492.5	cystathionine	255.8
threonine	113.4	phenylalanine	72.6
serine	326.9	β-alanine	379.8
glutamic	143.4	γ-aminobytyric	1797.0
glycine	114.4	ethanolamine	not quanti.
alanine	1086.1	ammonia	a lot
valine	-	lysine	-
methionine	-	arginine	693.0
isoleucine	189.0	proline (440)	186.6

* The concentration of amino acids is calculated in nmole amino acid/ 30 mg. of crude Fraction XIIIA

Study on Fraction XIIIB

Fraction XIIIB was a methanol insoluble fraction 25.43 g. (50.86% wt. by wt. of Fraction XIII). The chemical and ignition tests revealed that this fraction was mainly composed of chloride salts of Na, K and Mg.

The X-ray fluoresence spectrum of this fraction (Fig.191) exhibited that Fraction XIIIB contained P, Cl, K, Mn and Na.

The quantitative analysis of cations in Fraction XIIIB by using ICPS was performed and found that this fraction was mainly composed of Na, K and Mg: 72.46%, 13.28% and 11.90%, respectively.

The Ca, Mn, Fe and Cu were trace elements in this fraction.

3.16 Chemical Constituents of Fraction XIV

Fraction XIV was assigned for the exhausted plant after extraction with dichloromethane and 95% ethanol, successively. This fraction was further examined by extraction with 50% ethanol-water to yield a dark brown crude (6.75% wt. by wt. of dried plant material). The phytochemical screening test involving to tannin and polyphenol tests indicated that Fraction XIV was consisted of condensed and hydrolysable tannins. Moreover, Fraction XIV was also found to be an inorganic salt fraction. The major cations contained in this fraction were Na, Mg and K (53.46%, 31.07% and 12.22%, respectively), together with the Ca and Mn 2.53% and 0.73%, respectively. The Fe and Cu were present as trace elements in Fraction XIV.

3.17 Biological Activity Studies of Isolated Compounds

3.17.1 Compound 1

Compound 1 was isolated and elucidated its structure as a mixture of saturated long chain aliphatic hydrocarbons (C_{27}-C_{33}) from the leaves of R. apiculata Bl. Long chain n-alkane compounds are widely distributed in the plant kingdom as a component of the cuticular waxes which are common to the surfaces of the leaf, stem, flower and pollen (139). There had been only three major recent reports on the study of hydrocarbon in mangrove plants. One of which was on the leaves from several species cultivated in a shadehouse in Australia (140), the other were on Avicennia marina

from South Australia (141) and on the leaves of some Malaysian mangrove plants (139). However, there is no report involving to the biological activity studies of this substance.

From the bioassay results, Compound 1 was totally inhibited boll weevil feeding at dose level 5 mg. and also showed the antigrowth activity against the fungi Pythium ultimum and Helminthosporium teres (% T/C 120 and 12, respectively).

3.17.2 Compound 2 and Compound 3

Compound 2 and Compound 3, the major constituents of the leaves of R. apiculata, were isolated and identified as β-amyrin palmitate and a mixture of β-amyrinpalmitate and waxes, respectively. β-amyrinpalmitate and waxes always occurred as a mixture in the latex of various plant species, such as Balanophora abbreviata and B. indica (74,142).

From the bioassay results, Compound 2 showed the strongly feeding inhibition against the insect, boll weevil at dose level 5 mg. (100% inhibition), but it did not reveal the antifungal and the antibacterial activity.

3.17.3 Compound 5

Compound 5 was isolated from the leaves of R. apiculata and characterized its structure as β-amyrenonylpalmitate. The β-amyrenonylpalmitate was first isolated as a mixture of α- and β-amyrenonylpalmitate from the leaves of Carphenphous ordoratissimus (80) and there is no report about this compound furthermore. The biological activity of this substance did not mention in that paper. However, β-amyrenonol (the alcoholic part) had been reported to

reveal anticancer effects against cancer cell lines SK-OV-3 and CAMA-1 at dose level 100 μg/mL. (143).

The bioassay results of this compound showed the moderately antigrowth activity against the fungi Pythium ultimum and Helminthosporium teres with % inhibition 50 and 66, respectively. Moreover Compound 5 revealed the antibacterial activity against Xanthramonas campestrous with 50% inhibition. The bioassay evaluation of this compound on the insect antifeedant activity is in progress.

3.17.4 Compound 6 and Compound 16

Compound 6 was separated from the leaves of R. apiculata and elucidated its structure as a mixture of saturated long chain aliphatic primary alcohols (C_{30}-C_{36}), while Compound 16 was isolated and identified as a mixture of long chain alcohols (C_{28}-C_{32}) from the heartwoods of this plant. The saturated long chain aliphatic alcohols are widely distributed in the waxy fraction of the plant extract. The triacontanol ($C_{30}H_{61}OH$) had been reported to be widely used as a plant growth regulator (PGR) (144,145). 10-20 mg./L. of triacontanol used as the plant growth stimulating agent on tomato plants had been illustrated (146).

From the bioassay results, this compound revealed neither the antifungal nor the antibacterial activity. However, it showed the feeding activity to insect, boll weevil (% T/C 214).

3.17.5 Compound 7

Compound 7 was separated and identified as β-amyrin from the leaves of R. apiculata. β-amyrin belonged to oleanane

triterpene series which are the largest group of triterpenoid compounds. This compound was originally isolated in the nineteenth century from elemi resin both free and esters in rice embryo, gutta percha, grape seeds and alfafa (147). β-amyrin had been reported to display hemolytic properties (H_{50} 2.8 x 10^{-5} μM.) (148) and exhibited antiatherosclerotic and hypolipemic activities (149).

The evaluation of this compound for the antigrowth against fungi and bacteria and for the antifeedant against insect, boll weevil is in progress.

3.17.6 Compound 8

Compound 8, one of the major constituents of the leaves of R. apiculata, was separated and identified as lupeol. Lupeol, a pentacyclic triterpene belonging to a lupane group, was first isolated from the seeds of Lupinus albus (147). This triterpenoid was found to be one of the most widely distributed substances in mangrove plants (34). Lupeol was said to be effective in rheumatism and urinary infections (149). It also showed significant antitumor activity in Sprague rats against the Walker 256 intramolecular tumor system (5WA16) (150). Besides, lupeol had been reported to infect with the fungus Verticillium (151). Moreover, in recent years, the acetyl derivative of this compound (lupeolacetate) was found to be used as the inhibitor of incidence of stress-induced ulcers and decreased the incidence of gastric ulceration induced by pyloric ligation (152).

The bioassay evaluation of this compound against fungi, bacteria and insect, boll weevil is in progress.

3.17.7 Compound 9

Compound 9, one of the major components of the leaves of R. apiculata, was identified as taraxerol. Taraxerol, a pentacyclic triterpenoid belonging to a taraxerane group, was first isolated from the roots of Taraxacum officinale (153). This compound had been reported that it did not induce gastric ulceration, but inhibited the incidence of stress-induced ulcers (152).

From the bioassay results, Compound 9 displayed totally inhibition against the fungi Pythium ultimum (100% inhibition) and 40% inhibition against Rhizoctonia solani. Moreover, it revealed 97% inhibition against the insect, boll weevil at dose lovel 7 mg.

3.17.8 Compound 10 and Compound 18

Compound 10 and Compound 18 were separated from the leaves and the heartwoods of R. apiculata, respectively. These two compounds were characterized as a mixture of β-sitosterol, stigmasterol and campesterol. These co-occurrence of three steroids are widely distributed in the plant kingdom. The plant steroids were well known to be used as precursors for preparing steroid hormones. For instance, stigmasterol had been reported to be used as precursors to synthesize progesterone hormone (154). Moreover, the steroids had been proved to have a barely significant antihypercholesterolemic effect while exhibiting no obvious effect on the heart or liver (155).

From the bioassay results, this mixture of steroids displayed the antifeedant activity 79% inhibition at dose level 4.5

mg., but did not show effect on fungi and bacteria.

3.17.9 Compound 11 and Compound 12

Both Compound 11 and Compound 12 were isolated from the leaves of R. apiculata. Their structures were elucidated to be taraxeryl ester of p-coumaric acids in cis and trans geometric isomers, respectively. The trans isomer, careaborin or taraxeryl-trans-p-hydroxycinnamate, was first isolated from the leaves of Careya arborea (119) and there is no report on this substance furthermore. Throughout the chemical literatures, there is no report on the cis isomer one. Therefore, taraxeryl-cis-p-hydroxycinnamate was a novel naturally occurring ester of taraxerol. Both of these compounds had no report on biological actvity studies.

The bioassay results revealed that Compound 12 showed the weakly inhibition against the fungi Helminthosporium teres and Pythium ultimum with % T/C 33% and 28%, respectively. The insect antifeedant activity against boll weevil of Compound 12 is in progress. Unfortunately, Compound 11 was obtained in such a limit amount that its biological activity could not be evaluated.

3.17.10 Compound 13

Compound 13 was separated and identified as wallichenol. This compound, a member of a lupane triterpenoid series, was originally obtained from the reduction of lupenalylacetate in 1941 (156). The first isolation of this compound as a natural product was derived from Gymnosporia wallichiana in 1979 (124).

Compound 13 displayed as a broad spectrum antifungal agent against Pythium ultimum, Rhizoctonia solani and Helminthosporium teres with % inhibition 81%, 73% and 100%, respectively. The antifeedant activity evaluation of this compound against the insect, boll weevil is in progress.

3.17.11 Compound 14 and Compound 23

These compounds were separated from the leaves and the heartwoods of R. apiculata, respectively and found to be identical to each other in all respects. Their structures were elucidated to be β-sitosteryl-3-O- β-D-glucopyranoside. Literature surveys stated that this compound, an abundant sterylglycoside, exhibited an inhibitory effect on vascular permeability, antiulcerogenic and hemostatic effects (157). It was also reported that a mixture containing this substance as a major constituent showed antitumor activity against P-388 leukemia (158,159). Moreover, this substance revealed inflammatory activity and was said to be used as drug carriers.

Nevertheless, Compound 14 and Compound 23 did not reveal the antigrowth against fungi and bacteria and did not show the insect antifeedant activity, either.

3.17.12 Compound 15

Compound 15 was isolated from the heartwoods of R. apiculata and identified as a mixture of steroidal ester of β-sitosterol, stigmasterol and campesterol and long chain carboxylic acids, and waxes.

From the bioassay results, this compound did not exhibit the antigrowth activity against fungi and bacteria, but it gave the strongly feeding inhibition against the insect, boll weevil at dose level 4 mg..

3.17.13 Compound 17

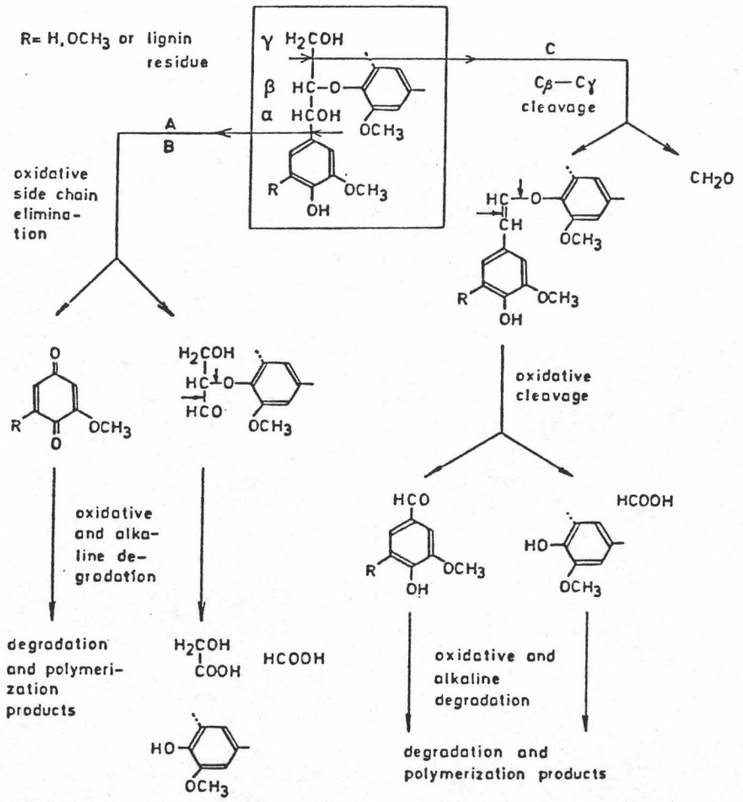
Compound 17, one of the major products of the heartwoods of R. apiculata, was separated and characterized its structure as a mixture of 11 saturated long chain aliphatic carboxylic acids. This type of compound always occur in variety of plant species (160). Nevertheless, there is no report concerned with the biological activity studies of this compound.

From the bioassay results, Compound 17 showed strong feeding inhibition against the insect, boll weevil at dose level 7 mg., but did not display the antifungal as well as the antibacterial activity.

3.17.14 Compound 19 and Compound 20

Compound 19 and Compound 20 were isolated from the heartwoods of R. apiculata. The former was identified as 2,6-dimethoxy-p-benzoquinone, while the latter was elucidated its structure as syringaldehyde. These two compounds were proved and suggested that both of them were derived from lignin degradation. The summary of the pathway of lignin degradation had been reported as shown in Scheme 3.21 (161).

Scheme 3.21 The summary of the pathway of lignin degradation (161)



The 2,6-dimethoxy-p-benzoquinone was known to occur in a wide variety of plants, i.e, genera Adonis, Euricoma, Acer, Betula, Marsdenia, Fraxinus, Xanthoxylum and the fungus Polyporous fumosus (162-167). However, there is no report of the occurance of this compound in Rhizophora genus. Literature surveys on the biological activity studies of 2,6-dimethoxy-p-benzoquinone

revealed that this compound was one of the most well-known bioactive ingredients. This compound showed a weak inhibition against the growth of gram-positive bacteria, Staphyloccocus aureus and Escherichia coli (minimum effective concentration: 62.5 and 100 μM, respectively) (168), and of the fungus Trichophyton interdigitale (minimum inhibition concentration: 300 M) (166). Antitumor activity of this compound was also observed against Ehrlich ascites carcinoma (168-169). Moreover, it displayed as an anticancer agent against P-388 lymphocytic leukemia test system in vitro (ED_{50} 0.038 μg/mL.) (167,170). Furthermore, this compound inhibited adenosine 3', 5'-cyclic monophosphate (c-AMP) phosphodiesterase activity (IC_{50} = 150 μM) (171). Oral administration of this compound to intact rats at a dose of 50 mg./kg. inhibited the tissue granulation induced by the substances implantation of a cotton pellet (166). Inhibition by this compound of platelet aggregation induced by arachidonic acid and collagen was reported at concentrations of 145 and 49 μM (IC_{50} values), respectively (164). 2,6-dimethoxy-p-benzoquinone had been obtained not only from natural product resources, but also from the synthetic methods. The action of nitrous acid on 1,2,3-trimethoxybenzene (171) and the hexacyanoferrate-catalyzed oxidation of 1,3,5-trimethoxybenzene with hydrogen peroxide (65) are two examples.

The syringaldehyde had also been reported to be a broad spectra biologically active compounds. To illustrate, it was said to be used as a plant growth regulator (172), an antimicrobial, an antifungal (173) and an antibiotic agents (174). Moreover, this compound was found to be the precursors for preparing an important

drug intermediate used in pharmaceutical industry, 3,4,5-trimethoxy benzaldehyde (175).

From the bioassay results, Compound 19 displayed the moderate antifungal activity against Pythium ultimum with 40% inhibition and the antibacterial activity against Xanthramonas campestrous with 64% inhibition. This compound also exhibited 89% feeding inhibition against the insect, boll weevil at dose level 6 mg.

Compound 20 revealed the antigrowth activity against both fungi and bacteria. The antifungal activity against the fungi Pythium ultimum and Helminthosporium teres with % inhibition 30% and 80%, respectively. The antibacterial activity against Xanthramonas campestros was also observed with 25% inhibition. The evaluation on the insect antifeedant activity of Compound 20 is in progress.

3.17.15 Fraction III, Fraction VII, Fraction X and Fraction XIV

These four fractions, the first two from the leaves and the rest ones from the heartwoods of R. apiculata, were proved to be hydrolysable and condensed tannin fractions. Tannins are common constituents of plant crude extracts and have been reported to have pharmacological actions on isolated smooth and cardiac muscles, and on blood pressure in animals (176). The condensed tannins administered orally exhibited antipeptic activity and stress-induced gastric lesions in mice (177). Moreover, tannins were said to be used as anticancer, antiviral and antimicrobial agents (178).