CHAPTER III

RESULTS AND DISCUSSION

The extraction of the roots of A. illicifolius with petroleum ether and 70% ethanol yielded petroleum ether soluble fraction (Fraction I, 0.8% wt) and 70% ethanol soluble fraction (24.8% wt). The crude product of 70% ethanol soluble fraction was partitioned between chloroform and water (3:1) giving chloroform soluble part (Fraction II, 1.21% wt) and water soluble part (23.2% wt). The crude product of aqueous fraction was extracted with methanol to afford a crude methanol soluble fraction (7% wt) and the insoluble product of methanol fraction (Fraction IV) (12.4% wt). Then crude methanol soluble fraction was extracted with chloroform-ethanol (8.5:1.5) yielding crude product of Fraction III 2.04% wt. (see Scheme I).

The results of separation and purification of crude Fraction I yielded three compounds which were assigned as Compound 1 m.p. 115°-116° (0.006% wt of dried roots), Compound 2 m.p. 77°-78° (0.056% wt of dried roots) and Compound 3 m.p. 167°-168° (0.08% wt of dried roots).

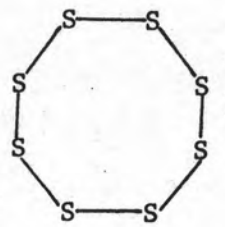
Four compounds were isolated from Fraction II purified, and identified as Compound 4 m.p. 137°-138° (0.094% wt of dried roots), Compound 5 m.p. 292° (decompose), Compound A m.p. 114°-115° and Compound B m.p. 146°-147°.

3.1 Structural Elucidation of Compound 1

Compound 1, m.p. 115°-116°, was isolated from crude Fraction I by quick column chromatography and recrystallized from petroleum ether, followed by methanol. This compound was slightly soluble in chloroform

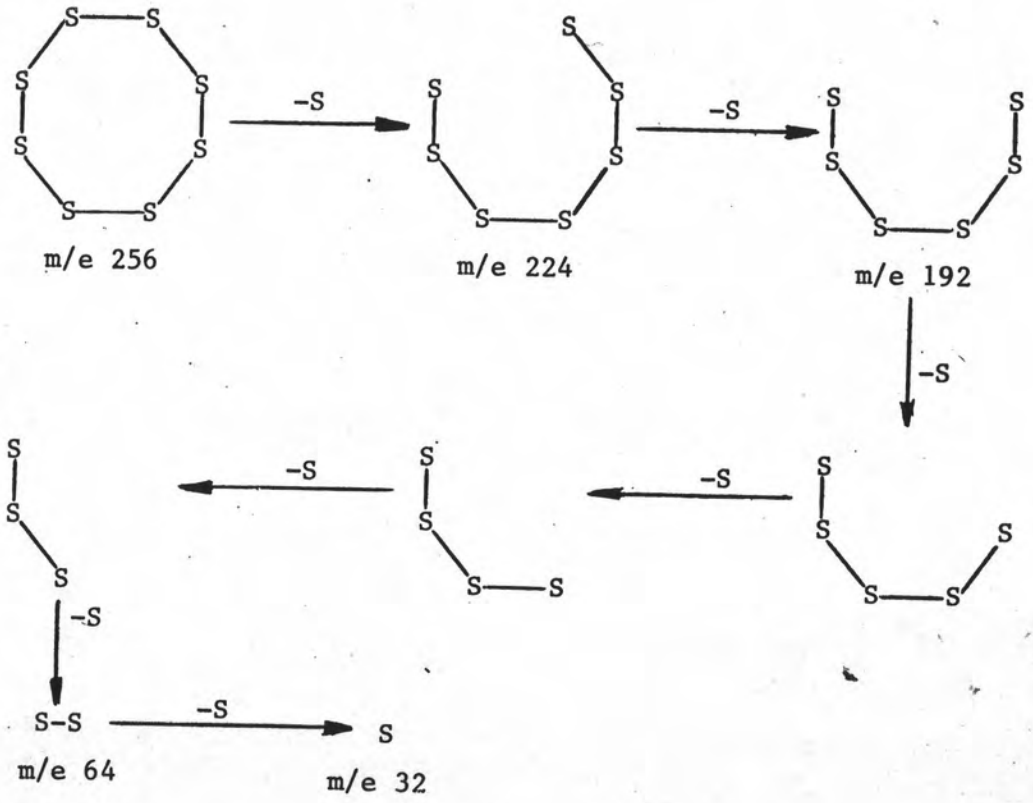
and insoluble in petroleum ether, methanol and water. The qualitative elemental analysis found only sulfur which indicated that this compound contained only elemental sulfur.

The mass spectrum showed the fragments at m/e 256 (M^+, parent peak), 224 (256-S), 192 (244-S), 160 (192-S), 128 (160-S), 96 (128-S), 64 (96-S) and 32 (64-S)[16]. The evidences from mass spectrum also indicated the possible skelaton for Compound 1 as the S_8 molecule which the fragmentation pattern of mass spectra of S_8 molecule was proposed in Scheme II. The result from the elemental analysis of this compound (see page 14) also supports that Compound 1 should be the sulfur molecule. Furthermore the IR spectrum showed a single absorption peak at 1380 cm^{-1} that identical with sulfur (S_8) molecule. The result of mixed melting point technique also confirmed that Compound 1 must be the sulfur (S_8) molecule.



Compound 1 S_8

Scheme II The proposed mechanism for mass spectrum fragmentation of Compound 1.



3.2 Structural Elucidation of Compound 2

Compound 2 was collected from separation of crude Fraction I by quick column chromatography using silica gel 60 as an adsorbent and eluted with 40% chloroform-petroleum ether. After recrystallization from hot petroleum ether gave Compound 2 as white amorphous solid, m.p. 77°-78° (Rf 0.260, solvent: 80% $CHCl_3$-P.E.).

The IR spectrum of compound 2 showed characteristic absorption bands which can be assigned on the next page.

Absorption peaks (cm^{-1})	Assignments
3200-3300	OH stretching
2960, 2850	asymmetric and symmetric C-H and methylene group (CH_2)
1460	C-H scissoring vibration of methylene
1260	twisting and wagging vibration of methylene
1000-1100	C-O stretching

The IR absorption spectra of this compound suggested the peak at 3200-3300 cm^{-1} was the signal of -OH functional group. The other IR absorption peaks also indicated that this compound containing only saturated hydrocarbon as the functional group.

The ^1H NMR spectrum of compound 2 showed the triplet at δ 3.63. This signal suggested that their was proton on the carbon which was adjacent the hydroxyl group. Another high intensity and sharp singlet peak at δ 1.27 that there were several interlinking of methylene groups in the molecule of this compound.

The molecular formula of this compound was proposed to be $C_{28}H_{56}O$. This formular was supported by elemental analysis informations and as well the mass spectrum data. The fragmentation of mass spectrum of this compound did not show the molecular ion peak at m/e 410 but one important peak was observed at m/e 392 which corresponded to the successive lose of one molecule of H_2O form the molecule and it showed other peaks at m/e 364 (392-$(CH_2)_2$), 336 (364-$(CH_2)_2$), 167 (336-$(CH_2)_{11}$-CH_3), 97 (167-$(CH_2)_5$) showed that the molecule of this compound composed of one methyl group, twenty seven methylene groups and a hydroxyl functional group.

The spectral informations confirmed that Compound 2 should be a longchain alcohol (see page 14), the physical and chemical properties, and acetate derivative of this compound suggested that this long chain alcohol must be an octacosyl alcohol which all evidence confirmed this conclusion[17]. The ^{13}C NMR spectrum of this compound displayed the signals of one carbon of CH_3 at 14.09 ppm, and α carbon of OH ($-CH_2-OH$) at 32.89 ppm, and one carbon of OH ($-CH_2-OH$) at 63.12 ppm which positively confirmed that Compound 2 was octacosyl alcohol. The structure was shown below.

$$CH_3-\overset{\alpha}{CH_2}-\overset{\beta}{CH_2}-\overset{\gamma}{CH_2}-(CH_2)_{21}-\overset{\gamma}{CH_2}-\overset{\beta}{CH_2}-\overset{\alpha}{CH_2}-OH$$

Compound 2 Octacosyl alcohol

3.3 Structural Elucidation of Compound 3

Compound 3, m.p. 167°-168°, was isolated from crude Fraction I with 44% chloroform-petroleum ether as eluents. This compound was purified by recrystallization in petroleum ether and in methanol to give white needles (Rf 0.23, solvent: $CHCl_3$). Compound 3 was reacted with Libermann-Burchard reagent giving a blue colour which indicate the present of the steroidal structure.

The IR spectrum of this compound revealed the OH stretching at 3300-3500 cm^{-1} and trisubstituted vinyl at 800 and 820 cm^{-1}. The sharp IR absorption peak at 1060 cm^{-1} suggest a C-O streching of alcoholic functional group.

The ^1H NMR spectrum exhibited a multiplet of olefinic proton at 5.33 ppm, doublet of doublet peak at 5.08 ppm which also indicated two olefinic protons of a ethylene groups that attached with two methylidine groups (-CH-CH=CH-CH-). A proton on that bearing hydroxy group showed a multiplet at 3.46 ppm and the protons of angular methyl groups

revealed peaks around 0.7-1.2 ppm[18]. The ^{1}H NMR spectra of this compound was also indicated that the molecular structure of this compound should be a steroid. When the ^{1}H NMR spectrum of this compound was compared to standard spectra of steroid, it showed that the spectra of this compound was similar to the ^{1}H NMR, spectra of stigmasterol[19]. These evidences confirmed that this compound was stigmasterol.

The MS displayed a molecular ion peak at M^{+} 412 (calcd. for $C_{29}H_{48}O$:MW 412), at m/e 394 which was corresponded to the cleavage of a molecule of H_2O from this molecule. The peak at m/e 273, indicated the loss of 139 from the parent mass and this suggested the removal of the molecule ($C_{10}H_{19}$), and it also showed peak at m/e 255 which involved the loss of H_2O and $C_{10}H_{19}$. Evidently this fragmentation process was common to a steroid such as[20].

Furthermore the ^{13}C NMR spectrum of Compound 3[21] revealed the signals which was identical to the signals of ^{13}C NMR spectra of stigmasterol which the chemical shift of each carbon atom was presented in Table VIII.

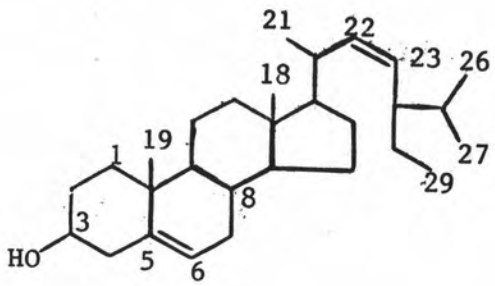
Table VIII ^{13}C NMR chemical shifts of Compound 3, stigmasterol and Compound 5.

Carbon	δ (ppm)		
	Stigmasterol	Compound 3	Compound 5
1	37.4	37.38	36.80
2	31.7	31.75	31.40
3	71.8	71.84	73.20
4	42.4	42.32	41.80
5	140.9	140.81	139.80
6	121.7	121.74	121.30
7	31.9	31.97	31.40
8	31.9	31.97	31.40
9	50.3	50.27	50.25
10	36.6	36.57	36.20
11	21.1	21.13	20.70
12	39.8	39.77	39.75
13	42.4	42.32	41.80
14	57.0	56.94	56.30
15	24.4	24.40	24.90
16	28.9	28.93	28.70
17	56.0	56.08	55.50
18	12.2	12.24	11.80
19	19.4	19.45	19.30
20	40.5	40.53	40.50
21	21.1	21.13	20.70
22	138.4	138.32	137.60
23	129.4	129.38	128.60
24	51.3	51.31	51.28
25	31.9	31.97	31.40
26	19.0	19.02	18.90
27	21.1	21.13	20.70
28	25.4	25.47	25.70
29	12.0	12.08	11.60

Compound 5 showed C_1^1 = 106.8, C_2^2 = 73.2, C_3^3 = 69.9, C_4^4 = 61.4, C_5^5 = 56.3 and C_6^6 = 56.2 ppm.

The data of UV, GC and mixed m.p. of this compound clearly confirmed that Compound 3 was stigmasterol.

According to the physic chemical properties of Compound 3, acetate as well as all spectrum data concluded that Compound 3 was stigmasterol.



Compound 3 Stigmasterol

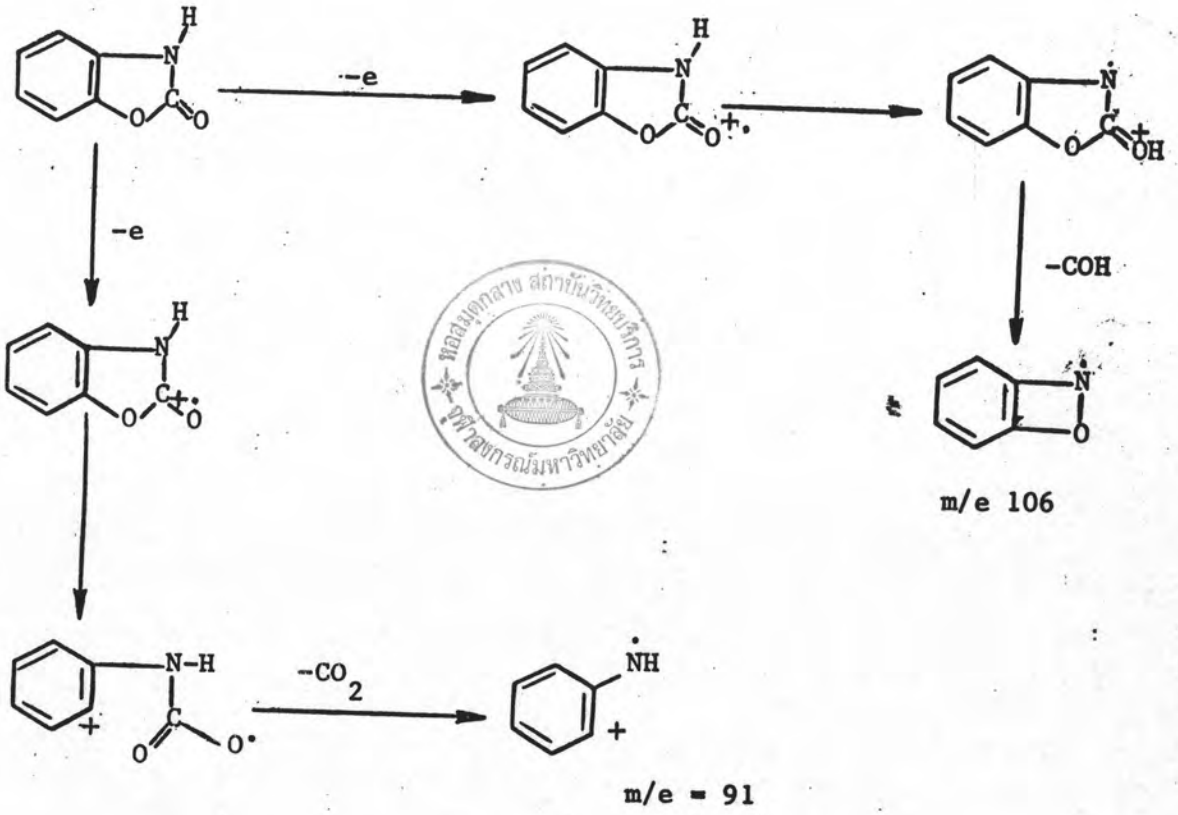
3.4 Structural Elucidation of Compound 4

Compound 4, m.p. 137°-138° was separated from 50% chloroform-petroleum ether by repeat column chromatography and purified by recrystallization from 5% chloroform-petroleum ether yielding a colourless rhombic 472 mg (Rf 0.314, solvent: 2% MeOH : $CHCl_3$). It gave positive tests with Dragendorff's and Kraut's reagents which was the characteristic of alkaloids. The quantitative elemental analysis for C, H and N, indicated a molecule formular of $C_7H_5NO_2$. The ^1H NMR spectrum of Compound 4 demonstrated broad singlet peak at δ 9.92, this was assigned to N-H (1 H) and showed singlet at δ 7.14 indicating 4 protons of aromatic ring.

The UV spectrum showed λmax at 202 nm (Σ 7000) manifisted the presence of aromatic ring in Compound 4.

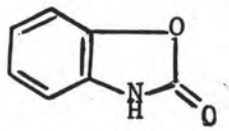
The MS showed mass fragments at m/e 135 (M^+, calcd. for $C_7H_5NO_2$: MW 135), 106 (M^+-COH), 91 (M^+-CO_2), 79 (M^+-C_2H_2NO), 63 (M^+-$C_2H_2NO_2$). The fragmention pattern was assigned on Scheme III.

Scheme III The proposed mechanism for MS fracmentation of Compound 4.



All evidences indicated that Compound 4 was benzoxazoline-2-one which was confirmed by ^{13}C NMR spectrum which showed the following signals: one carbon of carboxyl (CO) at 156.40 ppm, one α carbon adjacent to oxygen (-C-O-) at 143.94 ppm, one α carbon adjacent to nitrogen (C-N), a γ carbon of oxygen (-C-C-C-O-) at 124.20 ppm, one β carbon of nitrogen (-C-C-N-) at 122.74 ppm, one γ carbon of nitrogen (C-C-C-N-) at 110. 32 ppm and one β carbon of oxygen (-C-C-O-) at 110.15 ppm. It was also confirmed by

comparing other physic chemical properties, i.e., IR and ^{1}H NMR spectrum with synthetic benzoxazoline-2-one. Thus concluded that Compound 4 must be benzoxazoline-2-one[22].



Compound 4: Benzoxazoline-2-one

3.5 Structural Elucidation of Compound 5

Compound 5 was isolated from crude Fraction II by column chromatography using alumina as an adsorbent and the column was eluted with 20% methanol chloroform to afford white amorphous, m.p. 292° decomp, $|\alpha|^{23}$-48.5° (MeOH : $CHCl_3$ = 2:1) and Rf = 0.769 (solvent: 10% MeOH : $CHCl_3$). Compound 5 was reacted with Liebermann-Burchard to give a violet colour and positive test on Molish's reagent which was indicated that Compound 5 was a steroid or tripenoid glycoside.

The IR spectrum showed O-H stretching at 3400 cm^{-1}, and C-O stretching of glycosidic linkage at 1020-1080 cm^{-1}.

The ^{1}H NMR spectrum revealed the signals at: δ 0.66-2.00 ppm which corresponding to the methyl and methylene protons. Anomeric proton of sugar moiety showed the doublet peak (J = 8 H_2) at δ 4.2 ppm. and the other protons at carbon of sugar moiety gave the multiplet between δ 4.25-5.00 ppm, as well as the olefinic protons at δ 5.20-5.50 ppm[23].

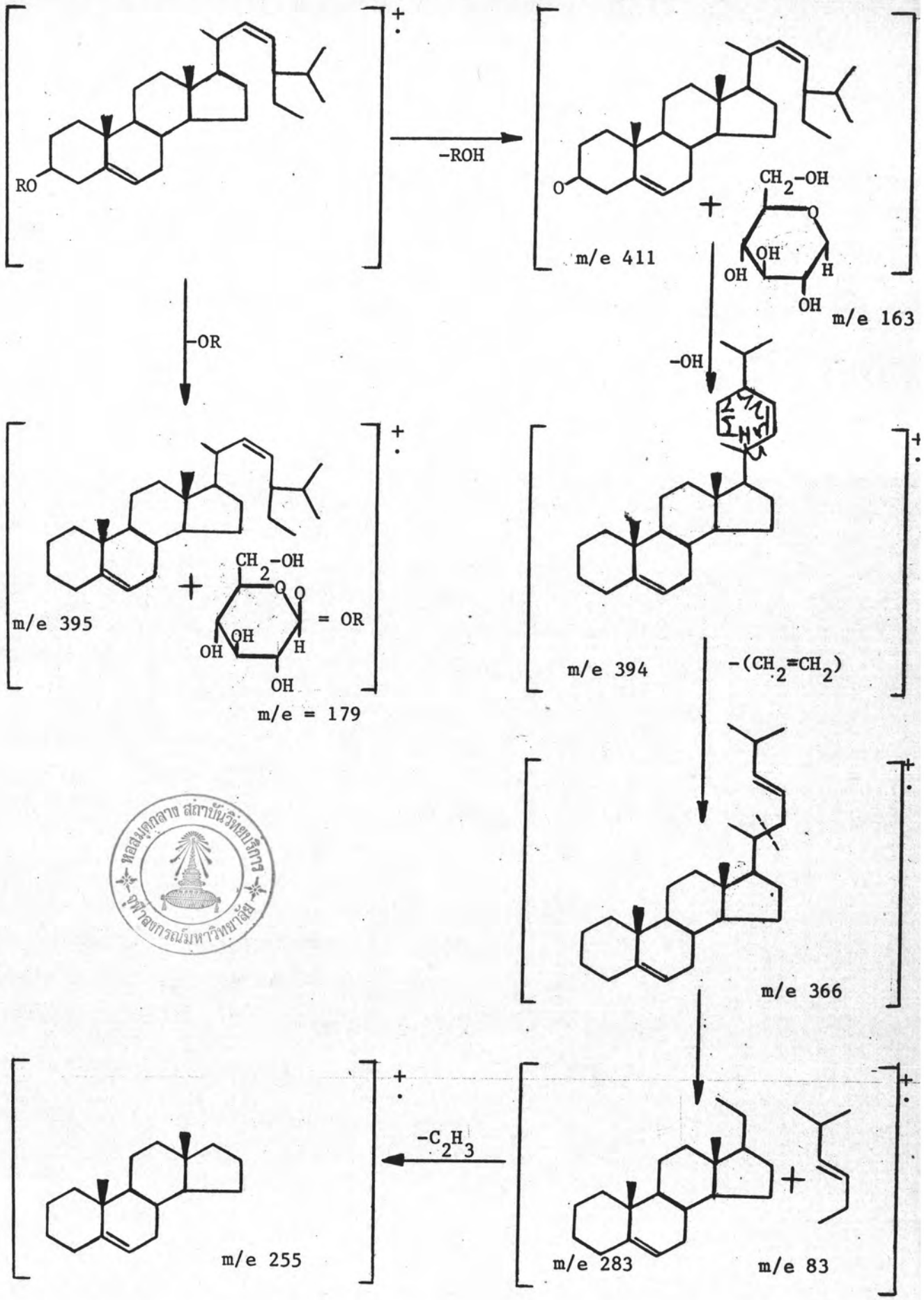
The ^{13}C NMR spectrum showed four signals of sp^2 carbons double bond at 139.8, 121.3 and 137.6, 128.6 ppm and the other six signals of the carbons which adjacent to the oxygen of the sugar moiety occurred at 106.8, 73.2, 69.9, 61.4, 56.3 and 56.2 ppm. The rest of

signals in this spectrum was similar to the spectrum of usual steroid compounds and assignment of the ^{13}C NMR spectrum shown in Table VIII.

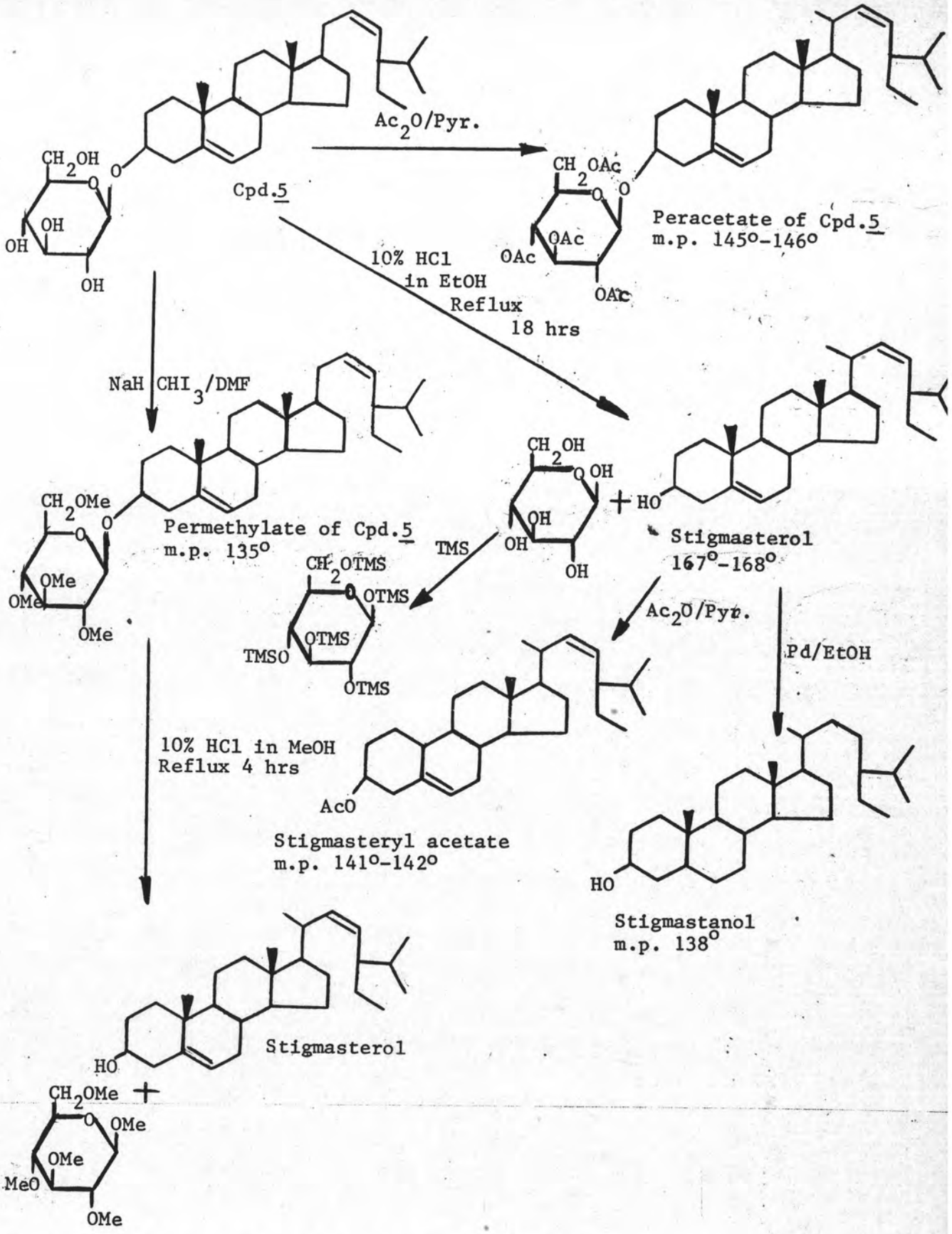
The UV spectrum gave max 203 nm (6084) which indicated that Compound 5 had double bond as a functional group.

The MS showed[24,25] mass fragments at m/e 412 (calcd. $C_{29}H_{48}O$: 412), 395 (412-OH), 394 (412-H_2O), 366 (394-C_2H_4), 283 (366-C_6H_{11}), 255 (283-C_2H_4), 179 ($C_6H_{11}O_6$), 163 ($C_6H_{11}O_5$) and 83 (C_6H_{11}). From the fragmentation in MS spectrum suggested that Compound 5 had a steroid nucleus and a sugar moiety which linked together as a steroid glycoside molecule. The mass fragmentation pattern of Compound 5 was presented in the following Scheme IV and the chemical reactions of Compound 5 were shown in Scheme V.

Scheme IV The possible mass spectrum pattern of Compound 5.



Scheme V Chemical reactions of Compound 5

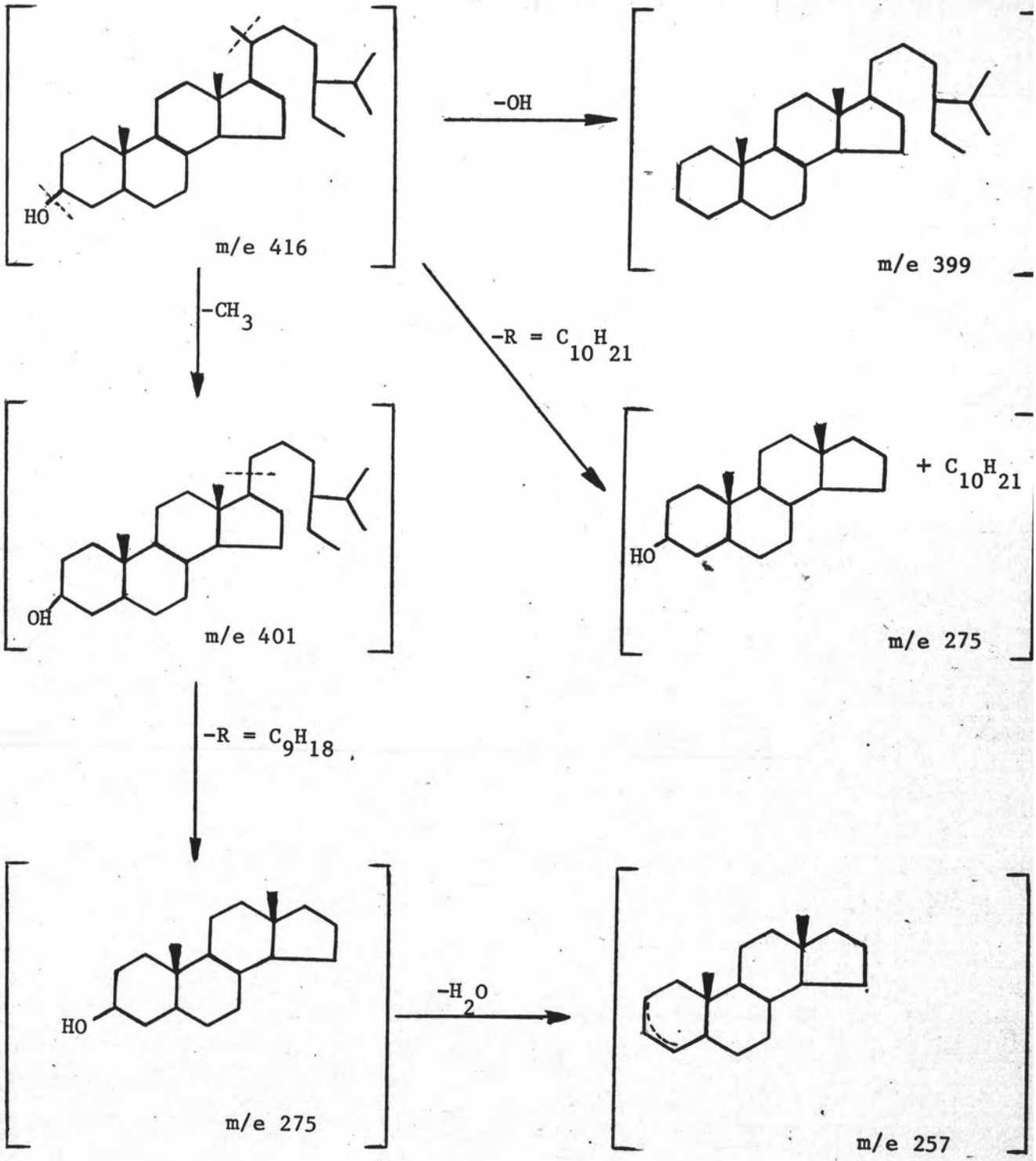


Acid hydrolysis of Compound 5 with 10% hydrochloric acid in ethanol and further diethyl ether extract gave aglycone as white needles, m.p. 167°-168° (Rf 0.23, solvent: $CHCl_3$) and $[\alpha]_D^{23} = -51.3°$ (in $CHCl_3$: MeOH = 2:1). The aglycone gave blue colour with Liebermann-Burchard reagent. The IR spectrum of aglycone and its acetyl derivative were identical with IR spectrum of authentic stigmasterol and the acetate of stigmasterol respectively. The GC analysis of aglycone gave Rt = 3.7 min, which is the same as Rt value of stigmasterol. The elemental analysis of the aglycone was corresponded with the value for stigmasterol.

Hydrogenation of aglycone gave white crystals, m.p. 137°-138° (Rf 0.714, solvent: 80% $CHCl_3$-P.E.). The IR absorption bands showed O-H stretching at 3400-3500 cm^{-1} and C-O stretching at 1050 cm^{-1} but the absorption peaks at 800-840 and 960-977 cm^{-1} of the carbon double bond were disappeared. The ^{13}C NMR spectrum of hydrogenated derivative was similar to the position of stigmasterol except that the peak of carbon double bond which was absented. The ^{1}H NMR spectrum of hydrogenated aglycone showed a broad multiplet signal at δ 3.59 which was related to one proton at C-3. The other signals in this spectrum were identical with the pattern of stigmasterol except that the signal of proton at carbon double bond which was disappeared at δ 5.32 and 5.05 (C-5, 6 and C-22, 23). The MS showed molecular ion peak at 416 (calcd. $C_{29}H_{52}O$: MW = 416) and other fragments at m/e 401 (M^+-CH_3), 399 (M^+-OH), 398 (M^+-H_2O), 383 ($M^+-H_2O-CH_3$), 273 ($M^+-C_{10}H_{21}$) and 257 ($M^+-C_{10}H_{21}-H_2O$). The fragmentation mechanism was assigned in Scheme VI. The GC analysis gave Rt value which was identical with stigmasterol (Rt = 30.62 min). The above data confirmed that derivative of aglycone was stigmastanol.

According to physical properties, spectral data of acetylated and hydrogenated derivatives of aglycone gave evidence supporting aglycone of Compound 5 was stigmasterol.

Scheme VI The possible mass spectrum pattern of stigmastanol



Characterization of the Carbohydrate Moiety

The hydrolysate from Compound 5 was determined by paper chromatography and TLC which showed a spot that identical with an authentic glucose. The trimethylsilylated derivative of the sugar was subjected to GC and showed the peak at Rt = 14.2 min which was in good agreement with the authentic penta-trimethylsilyl-β-glucopyranoside. The above result indicated that there was only β-glucose in the hydrolysate.

All evidences suggested that the Compound 5 was a glycoside which composing of β-glucose and stigmasterol.

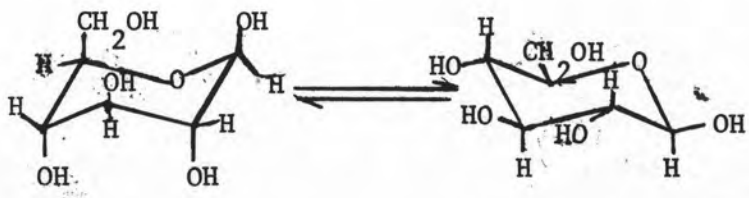
Permethylation of Compound 5[15]

Permethylation of Compound 5 by Brimacombe's method gave white needles, m.p. 135° (Rf = 0.690, solvent: 80% $CHCl_3$: P.E.), followed by methanolysis of this crystals product gave methyl-2,3,4,6-tetra-O-methyl-β-D-glucopyranoside as the product which was analyzed by GC. The result confirmed that glucose was attached to the hydroxyl group of stigmasterol molecule.

Peracetylation of Compound 5

Peracetylated derivative of Compound 5 was prepared as a white amorphous, m.p. 145°-146° (Rf 0.261, solvent: $CHCl_3$). The IR spectrum showed absorption peak of carbonyl stretching of acetoxy group at 1760 cm^{-1} and C-O stretching at 1220-1260 cm^{-1}. The remainder of this spectrum was similar to the IR spectrum of Compound 5 except that the O-H stretching was absent. The ^{13}C NMR spectrum showed signals of four carboxyl of acetoxy groups (4 $OCOCH_3$) at 169.2, 169.3, 170.3 and 170.6 ppm, four carbons double bond of C-5, 6 and C-22, 23 at 142.3, 122.1, 138.2 and 129.3 ppm and the six carbons of a sugar moiety showed the signals at 99.6, 71.7, 72.9, 68.9, 71.5 and 62.1 ppm of C_1', C_2', C_3', C_4', C_5' and

C_6' respectively. This data supported that Compound 5 was composed of six carbon atoms in the sugar moiety. The ^1H NMR spectrum revealed proton of acetoxy group ($OCOCH_3$) at δ 2.02-2.03 and anomeric proton of sugar at δ 4.65 (1 H, d, J = 7.5 Hz). From the last chemical shift and coupling constant (J = 7.5 Hz) indicated the β glycosidic linkage[26,27]. The β-glycosidic linkage, has two possible chair conformations as $^4C_1$ (D) : J = 7-10 Hz and $^1C_4$ (D) : J = 2-4 Hz which was an anomeric proton and proton at C-2 are diaxial and diequatorial respectively. The observed value of coupling constant (J = 7.5 Hz) is in agreement with $^4C_1$ (D) chair conformation of β-D-glucopyranoside[27]. The two possible chair conformations of β-D-glucopyranoside were shown below.



$^1C_4$ (D) $^4C_1$ (D)

The method of free energy calculation is considered for conformational analysis. Because of the $^4C_1$ (D) conformation has lower free energy (2.05 Kcal/mole), of course a more stable one (Table IX)[28]. The free energies of two chair forms differ by 5.95 Kcal/mol; this result confirmed that the β-D-glucopyranose adopted $^4C_1$ (D) conformation.

Table IX Predominant conformation of D-aldopyranose in aqueous solutions.

Aldose	Conformation found by ^1H NMR	Conformation found by Calculation	Calculated interaction energies (K cal/mole) $^4C_1$	$^1C_4$
β-D glucose	C_1	C_1	2.05	8.0

Conformational analysis of carbohydrate component in Compound 5 by Klyne method[29].

Klyne demonstrated that in the steroid glycoside, the rotation contribution of the carbohydrate component (ΔC) is almost independent on the nature of steroid component.

Molecular rotation : $[M]_D = [\alpha]_D \times$ mol. wt./100.

$\Delta C = [M]_D$ of steroid glycoside $- [M]_D$ of free steroid.

The rotation contribution of the carbohydrate (ΔC) is very approximately equal to $|M|_D$ of the corresponding α- or β- methylglycopyranoside.

From data;

Compound 5 found, $[\alpha]_D^{23} = -48.5^o$

MW. = 574

Aglycone as stigmasterol showed, $[\alpha]_D^{23} = -51.3^o$

MW. = 412

$\Delta C = [M]_D$ of Compound 5 $- [M]_D$ of stigmasterol

$$= \frac{-48.5^o \times 574}{100} - \frac{-51.3^o \times 412}{100}$$

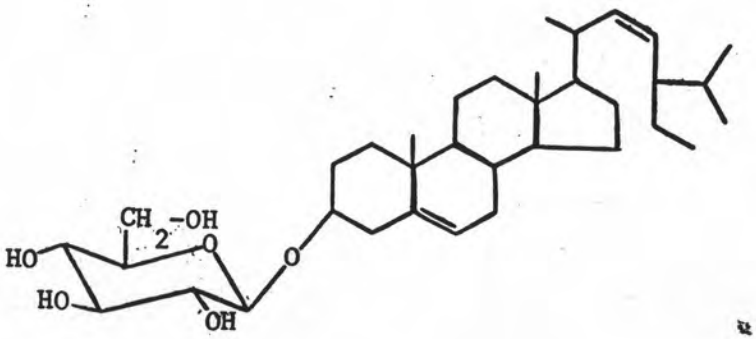
$$= -278.19^o + 211.356^o$$

$$= -66.834^o$$

The calculated molecular rotation of sugar, -66.834^o, which corresponding to methyl -β-D-glucopyranoside ($[M]_D = -63$). This method supported that the sugar of Compound 5 was β-D glucopyranoside.

From spectral evidence and some chemical reactions confirmed that non-aqueous part was stigmasterol, and an aqueous part was β-D-glucopyranose. Peracetylation of Compound 5 clearly suggested that the glucose unit in the glycoside was β-D glucopyranose which was attached at C-3

of stigmasterol. The above assignments of the glycoside linkage in the Compound 5 was also supported by the calculated molecular rotation (M_D) value for this structure which was in good agreement with the experimental value. Therefore, the exact configuration of the sugar linkages in the glycoside was β-D-glucose. Hence the Compound 5 was stigmasteryl-β-D-glucopyranoside. The structure of Compound 5 were showed as follow:



Compound 5 Stigmasteryl-β-D-glucopyranoside

3.6 Structural Investigation of Compound A

Compound A, m.p. 114°-115°, was isolated by repeating column chromatography and recrystallization from 10% methanol-chloroform to afford white amorphous (Rf 0.675, solvent: 5% MeOH-$CHCl_3$). The IR spectrum revealed that this compound had O-H, N-H and C=O groups at 3350, 3400 and 1675 cm^{-1}, respectively. The ^1H NMR spectrum showed only one peak at 1.26, suggesting poly-methylene groups in this compound A. The ^{13}C NMR spectrum revealed the C=O at 186.84, 108.06 and the others of chemical shift which was the peak of poly-methylene group. The MS showed the mass fragments at m/e 492 (M^+) 464 (M^+-CO), 451 (464-CH_3), 423 (451-$(CH_2)_2$), 394 (423-COH), 339 (357-H_2O), 308 (339-CH_3O), 278 (308-CH_4N). The acetylated derivative of Compound A, m.p. 88°-89°,

gave one spot on TLC which suggested the present of hydroxyl group in Compound A and IR spectrum showed N-H stretching at 3450 cm^{-1} and, carboxyl of acetate group at 1750 cm^{-1}. These results confirmed that Compound A composed of N-H, C=O and OH as its functional groups. But Compound A can not be identified since it was slighly soluble in the solvent and obtained in only small amount.

3.7 Structural Investigation of Compound B

Compound B, m.p. 146°-147°, was recrystallized from methanol yielding white amorphous (Rf 0.483, solvent: 5% MeOH-$CHCl_3$). The IR spectrum showed N-H stretching at 3350 cm^{-1}, O-H stretching at 3200 cm^{-1} and C=O stretching at 1620 cm^{-1}. The ^1H NMR spectrum exhibited signals of strong peak at δ 1.27 which was proton of poly-methylene proton, and other signals at 3.50 and 3.80. The ^{13}C NMR spectrum showed C adjacent to O at 61.39, 72.28 and 72.65 ppm (C-OH) and two carbon atoms of carboxyl group (C=O) at 176.08 and 202.09 ppm and carbon atoms of poly-methylene occurred at 14.14, 22.92, 25.47, 26.11, 29.58, 29.96, 32.18, 33.16 and 34.78 ppm. The MS gave mass fragments at m/e 482 (M^+), 453 (M^+-COH), 439 (M^+-CH_2-COH), 408 (425-OH), 339 (357-H_2O), 278 (308-CH-OH), and 111 ($C_{11}H_{23}$). Compound B was acetylated to afford white needles, m.p. 61°-62°. Its IR spectrum showed N-H stretching at 3400 cm^{-1} and carbonyl of acetoxy group at 1730 cm^{-1}. The ^1H NMR spectrum displayed protons of acetate group ($OCOCH_3$) at 2.01, 2.09 and 2.08 ppm. All the above data suggested that Compound B had N-H, OH and C=O as its functional groups. The structure of Compound B was not completely elucidated because of the limited amount of this compound.

3.8 Pharmacological Uses of Isolated Compounds

Sulfurs is widely used as external medicine in the form of oilment for skin diseases such as scabies, ringworm and favus[30] and also used

in other diseases, e.g. Gramulomatus Rossacea and fugicide[31]. The oilment for fungicide of sulfur composed of 3% precipitate of sulfur and 3% salicylic acid in petrolatum[32]. The isolation of sulfur from this plant was done by petroleum ether extraction which had dirrerent solubility from the pure compound because of the modification of other constituents the plant. This effect of solubility may bring about more medicinal activity of this plant than pure sulfur.

Benzoxazoline-2-one was a heterocyclic compound which have been investigated extensively primarily for their medicinal value as central nervous system depressants which exhibit analgesic, antipyretic, anticonvulsant, hypnotic and skeleton muscle relaxant activity[33]. It also used in resistance factor for fungi. Fusarium nivale, Advani and Sam[34] reported the preparation of a number of ribose derivatives of benzoxazoline-2-one for their evalution as anticancer and antiviral agents. So this compound may be the biological active agent of this plant.

Stigmasterol was also proved to had a barely significant antihypercholeslerolemic effect while exhibiting no obvious effect on the heart or liver[35].