CHAPTER 5

STRUCTURE ELUCIDATION OF ISOLATED COMPOUNDS

5.1 Introduction

NMR data was obtained by Mr NF Makhubela at the Department of Chemistry, MEDUNSA and the Mass Spectra were obtained by Dr. Boshoff at Cape Technikon. Dr DR. Katerere assisted with the interpretation of the information.

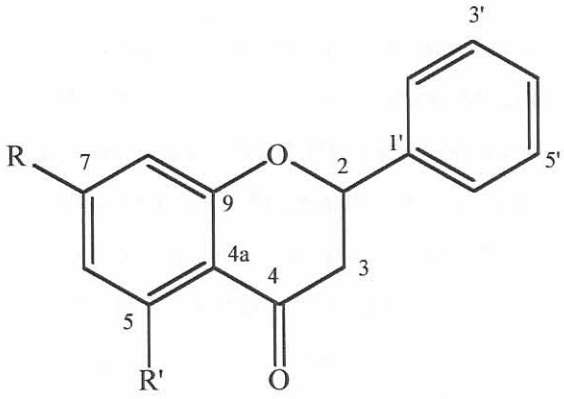
5.2 Overview of spectra of all compounds

The NMR spectra of AS-1, AS-13b, AS-130 and AS-164 are similar and are typical of flavonoids. They possess 2-phenyl chromanone as the parent skeleton. The heterocyclic ring has three carbon resonances, namely; oxymethine (C-2); resonating between 71.3 - 80.3 ppm, aliphatic methylene (C-3); resonating between 39.5 - 46.4 ppm, and carbonyl (C-4); resonating between 186.4 - 198.5 ppm (Agrawal, 1989). C-2 of the molecules is a centre of asymmetry and two forms of each structure are possible. However most of the naturally occurring flavanones acquire phenyl substituent at C-2 position in the pseudo-equatorial position. They bear a hydroxyl substituent at C-5 and C-7 positions hence the assignments of carbon resonances of ring A in case of 5,7-dihydroxyflavanone are of significance importance (Wagner *et al,* 1976). The carbonyl resonance (C-4) depends on the presence or the absence of the *para*-substituted C-5. In case of 5-unsubstituted flavanones C-4 resonance absorbs between 189.7 - 191.7 ppm except for the 7,8,3`, 4`-tetrahydroxyl flavanone, where it resonates at appreciably low field position δ194.5. In 5-hydroxylated flavanones, C-4 absorbs at deshielded position 195.6 - 197.3 ppm because of hydrogen bonding (Agrawal, 1989). The chemical shift of C-3 is independent of the substituents in the aromatic rings and should there be a shift it will be upfield.

1-2 ppm in 5-hydroxylated flavanones compared to the 5-unsubstituted.

All the six carbons of ring-A in flavanones do not superimpose with each other hence give rise to six signals unless there is symmetry.

Based on the spectra, two flavanones and one chalcone were isolated from the leaves of *C. apiculatum* Fig.4.24. The structures were elucidated by NMR and confirmed by spectrometry section. 3.



Compounds	R	R'
AS-130	OH	OH
AS-13b	OMe	OH

Fig. 5.1 The basic structure of flavanones isolated in this study

Table 5.1. Spectral data of flavanones isolated in this study.

	AS-130[x]		AS-13b[y]
Carbon position	**^{1}H resonance**	**^{13}C resonance**	**^{1}H resonance**
1	-	-	-
2	5.33 (dd, J= 3.0; 13.1)	79.5	7.80 (d, J=15)
3	2.71 (dd, J=3; 17.1) 2.98 (q, J=3.9; 15.2)	43.7	3.41 (q, J=1.8; 7.5) 8.26 (d, 15)
4	-	196.2	-
4a	-	103.6	-
5	-	164.8	-
6	5.96 (s)	97.2	6.04
7	-	164.9	-
8	5.96 (s)	95.9	5.99
9	-	163.6	-
1'	-	138.7	-
2'/6'	7.34 (m)	126.5	7.69 (m)
3'/5'	7.34 (m)	129.3	7.45 (m)
4'	7.34 (m)	128.8	7.27 (m)
7-OCH_3	-	-	3.82 (s)

[x] Spectra obtained in d-chloroform, [y]Obtained in d-acetone; ^{13}C not done
J is the coupling constant in Hz.

5.3 Structure of compound AS-130

About 4.7 mg of AS-130 was isolated as a cream-coloured compound. High Resolution Electron Impact Mass Spectroscopy (HREIMS) gave the molecular ion M^+ at *m/z* 256 corresponding to $C_{15}H_{12}O_4$. The base peak was seen at *m/z* 256 $[M-H]^+$. Other prominent peaks appeared at *m/z* 179 (67%) $[M-C_6H_5]^+$ and 167 (28%) $[M-C_7H_5]$. The fragmentation is typical of flavanones as illustrated by fig.4.23. ^1H-NMR spectra show a double doublet at 2.71 ppm (J = 3, 17.1 Hz), a quartet at 2.98 ppm (J = 3.9, 15.2 Hz), another double doublet at 5.33 ppm (J = 3.0, 13.1 Hz). Each integrates to one proton and is typical of the H-2 and H-3 *cis-trans* protons in a flavanone moiety. The signal at 5.96, a singlet integrating to two protons as well as the complex multiplet at 7.28 – 7.40ppm confirm the suspicion that this is a flavonoid with a mono-substituted C-ring. The signal at 5.96 ppm is typical of the H-6/8 protons in ring-A, implying that C-5 and C-7 have hydroxyl substituents. ^{13}C-NMR shows signals typical of an aromatic ring (126 – 129 ppm). The signal at 196.2 ppm is due to the ketonic carbon, C-4, which characteristically resonates between 186 and 199 ppm (Agrawal, 1989). In this case it shows up near the extreme downfield end because of the deshielding effect of hydrogen bonding with the hydroxyl group attached at C-5. C-6 and C-8 resonate at 97.2 and 95.9 ppm, while the signal at 103 ppm is due to C-4a. These signals are typical of flavonoids. In most cases, the C-4a signal is small (or absent all together) (Katerere, 2001) as was seen later on with AS-1. This appears to depend on the relaxation time set during the acquisition mode on the machine.

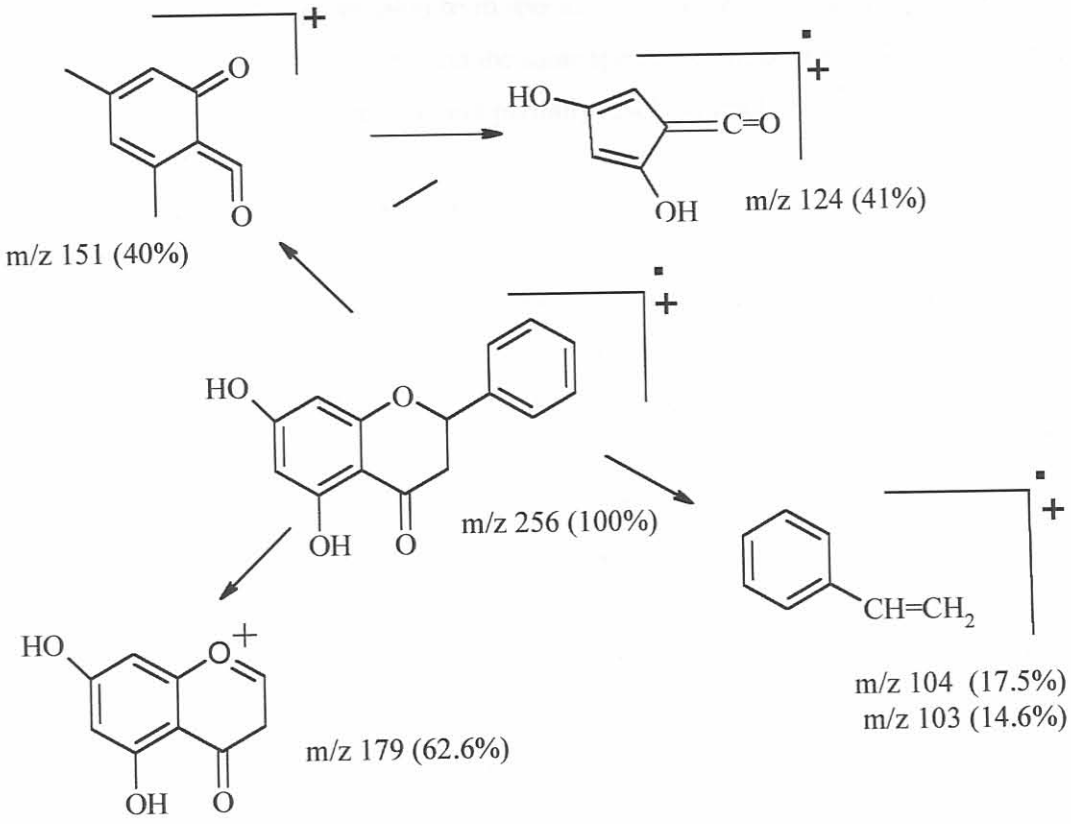


Fig 5.2 The fragmentation pattern of AS-130, which is typical of flavanones.

Based on the MS and NMR data as well as comparison with the literature (Agrawal, 1989), AS-130 was characterized as 5,7-dihydroxyphenyl flavanone, with commonly called pinocembrin.

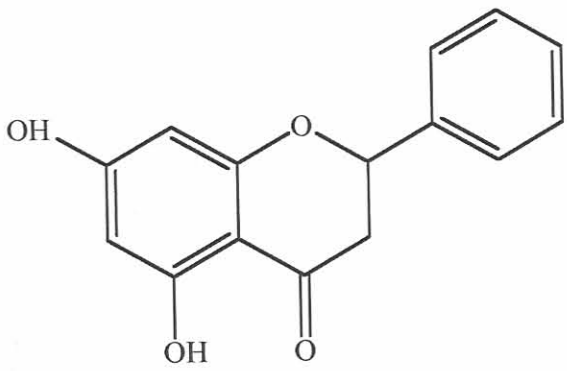


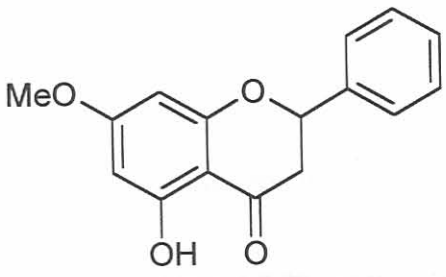
Fig.5.3.Chemical structure of Pinocembrin

Pinocembrin has also been isolated from species of *Alnus; Pinus; Eucalyptus* and *Populus*, (Itokawa, 1981). Fraction AS-164 had the same spectroscopic data as AS-130 and appears to be the same compound isolated from different partially resolved fractions.

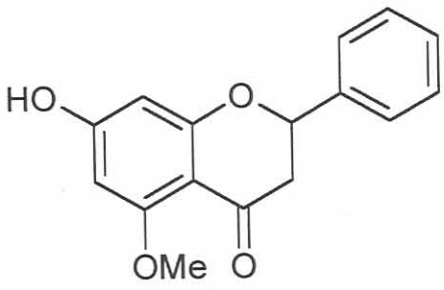
5.4. Structure of compound AS-13b

AS-13b (5.9 mg) was isolated as a white crystalline solid. HREIMS showed molecular ion M^+ at *m/z* 270,1 implying the molecular formula to be $C_{16}H_{14}O_4$. Other prominent peaks appeared at 167 $[M\text{-}C_8H_9]^+$ and at *m/z* 193 $[M\text{-}C_6H_7]^+$. 1H-NMR showed two doublets at 8.26 and 7.80 ppm, both with a coupling constant, J = 15 Hz signifying that these protons are oriented *trans* to each other. Their *cis* partner is at 3.41 (q, J = 1.8; 7.5). Together with the multiplets below 7.00pp, the singlets at 5.99 ppm and 6.04 pp (H-6 and H-8), the ^{1}H-NMR spectrum points to a flavonone. The region around 4.00 ppm shows two singlets implying that there are two methoxyl substituents. However the MS data supports the presence of only one showing the molecular ion to be at *m/z* 270 rather then 284. It could be suggested that the other peak may be due to an impurity whose other signals appear upfield i.e. a relatively non-polar contaminant.

Based on the MS and ^{1}H-NMR, two possible structures were were proposed,5-methoxy-7-hydroxy flavanone (I) and 5-hydroxy-7-methoxy flavanone (II). ^{1}H-^{1}H COSY (relayh) was performed. This showed cross-peaks between the methyl group at 3.82 ppm and both H-6 (6.04 ppm) and H-8 (5.99 ppm) implying that the methyl function is attached to C-7 rather than to C-5. With this data it was concluded that structure represented as (I) was more plausible than structure (II) because of the symmetry.



I



II

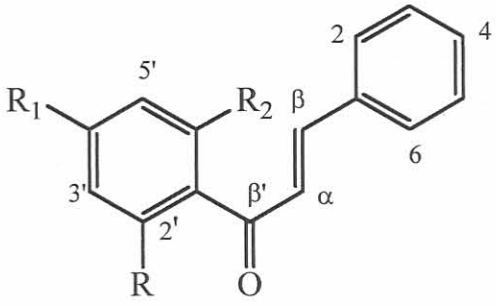
5.4. Possible structures of AS-13b.

AS-13b was elucidated as alpinetin. Alpinetin is one of the four flavonoids which were previously isolated from the crude extract of *Boesenbergia pandurata*. Its chemical structure was characterized by means of physical properties and spectroscopic data, and its antibacterial activity was apparently determined but no data is available (Tip-pyang, 2000). It was also isolated from *Mikania micrantha* (Jiang, 2001).

A number of other related flavanones have also been observed from Guinea *Piper* species, and it is entirely possible that at least two of them might have arisen through a chemical modification of chalcones as a consequence of isolation. Alpinetin is related similarly to alpinetinchalcone. Both of these compounds, regardless of their origins within the isolation procedures employed, represent several tenths of a percent of the total plant extract of Guinea *Piper* species (Alexander, 2001).

5.5 Structure of compound AS-1

About 7.2 mg of AS-1 was isolated as a yellow crystalline solid. HREIMS, showed the molecular ion M^+ at *m/z* 270 implying the molecular formula to be $C_{16}H_{14}O_4$. Prominent peaks appeared at *m/z* 167 $[M\text{-}C_8H_7]^+$ (52.3%) and the base peak at *m/z* 193 $[M\text{-}C_7H_7]^+$. Fig. 4.27. H-NMR spectrum shows a peak at 3.97 due to one O- methyl group. The peaks at 6.04 ppm (J = 2.1Hz) and 5.99 (2.1 Hz) are typical of H-6 and H-8 in flavonoids. In this case they are showing *meta*-coupling to each other. The complex multiplets at 7.27- 7.69 ppm are due to the mono-substituted ring B of the flavonoid.



Fig, 5.5. The basic structure and numbering of chalcones

The two doublets at 7.80 and 8.26 (J = 15.6 Hz) are alkenic protons which are deshielded by the conjugation arising from the phenylic ring on one side and the phenoyl group on the other side. Normally these protons appear between 5 - 6 ppm.

Table 5.2 Spectral data of chalcone isolated in this study

	AS-1	
Carbon position	**^{1}H resonance**	**^{13}C resonance**
α	8.26 (d, J = 15.6)	128.4
β	7.80 (d, J=15.6)	142.6
β'	-	193.1
1'	-	*
2'	-	164.4
3'	5.99 (d, J = 2.1	96.0
4'	-	166.0
5'	6.04 (d, J = 2.1)	92.2
6'	-	168.9
1	-	136.4
2/6	7.69 (m)	129.2
3/5	7.43 (m)	130.3
4	7.27 (m)	129.8
6'-OCH_3	3.97 (s)	56.4

The ^{13}C NMR spectra are typical of flavonoids and generally similar to that of AS-130. It shows a methoxyl group (56.4 ppm), the C-3'/ 5' signals at 92.2 and 96.0 ppm respectively, aromatic carbons at around 130 ppm, and the carbonyl at 193.1 pp (C-β'). In this case it is upfield relative to the similar group (C-4) in AS-130. This may be indicative of loss of hydrogen bonding. The signal expected at around 103 ppm is very small and almost indiscernible, again probably due to poor relaxation.

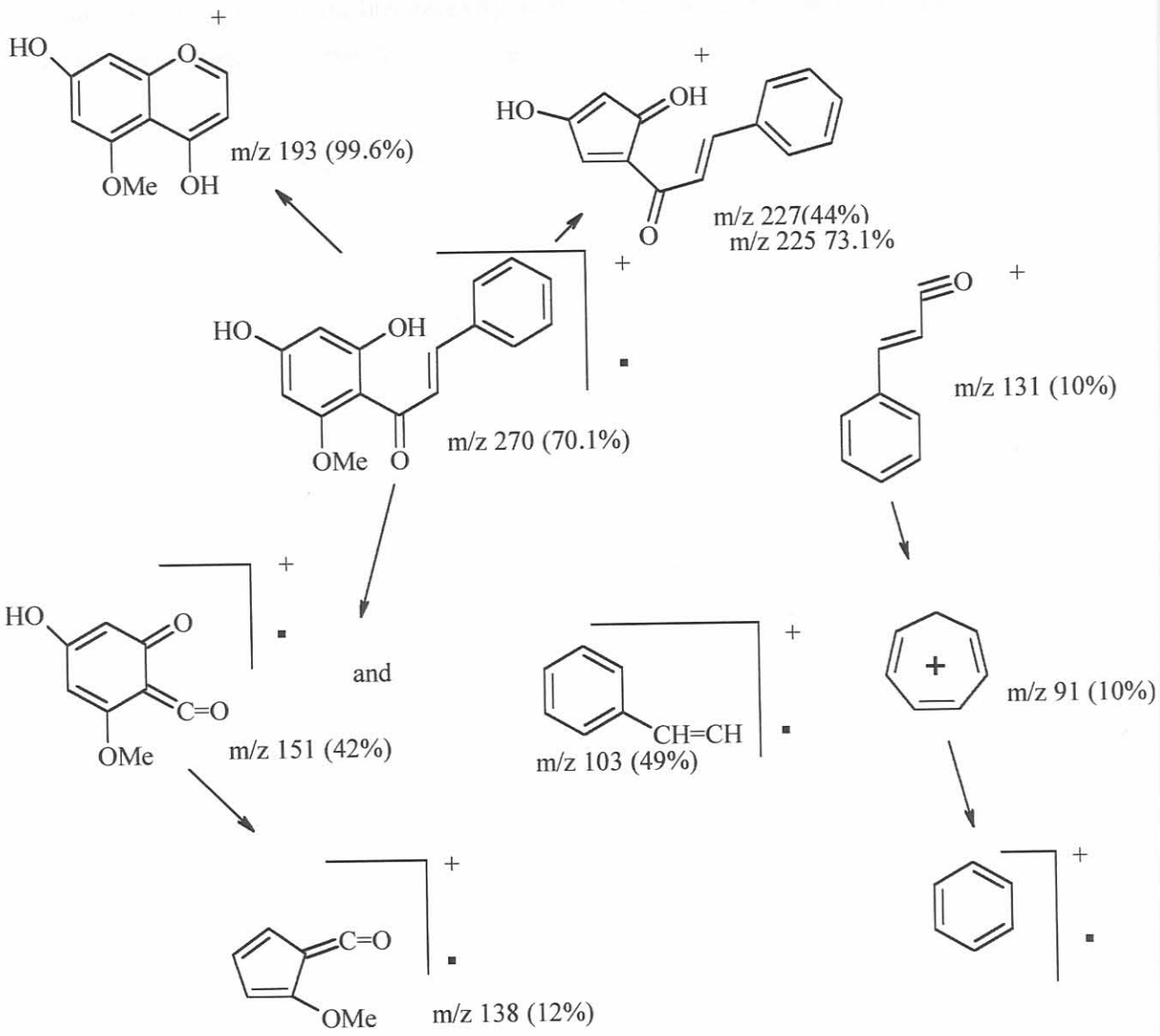


Fig.5.6. Typical fragmentation pattern of chalcones

From the NMR and MS obtained in the study, and by comparison with the literature (Wollenweber and Siegler 1982, Nascimento and Mors 1972; Agrawal, 1989; Katerere, 2001), AS – 1 was characterized as 2', 4' – dihydroxy – 6' – methoxychalcone. This is the chalcone of alpinetin (AS-13b) (which was also isolated in this study) and is known as cardamomin. Cardamomin has been used as a spice and as a flavouring agent in other medicines. The ancient Egyptians chewed it to whiten their teeth and also to sweeten their breath. In India it has found use in the cure of urinary and skin complaints. It has been previously isolated from *Boesebergia pandura, Pityrogramma*

chrysophylla, and species of *Alpinia, Piper and Populus* (Itokawa *et al*, 1981). The NMR data were considerably similar to those in the literature (Agrawal, 1989). This is only the second that this chalcone is being reported from the Combretaceae.