

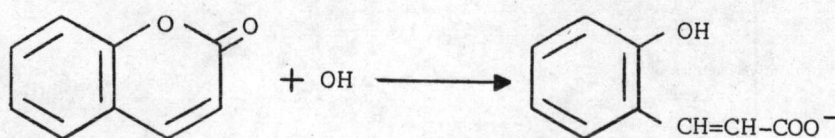
## Chapter IV

### Discussion

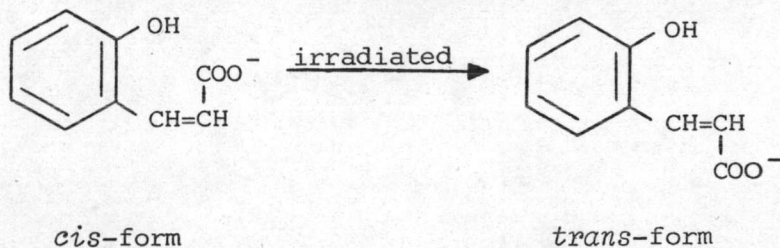
#### Discussion on The Extraction Procedure

Coumarins and flavonoids could be extracted from *Ageratum conyzoides* leaves by chloroform. Furthermore, lead acetate could precipitate some impurities such as phenolic compounds (tannins, flavonoids containing hydroxyl groups etc.), pigments, alkaloids, proteins etc., allowing coumarins and some flavonoids to remain in solution.

Coumarin dissolves in caustic alkali with cleavage of the pyrone ring and formation of alkali salts of *o*-hydroxycinnamic acid or their anions.



These solutions give a yellowish-green fluorescence within a few minutes when irradiated with long wave UV light. It is logical to assume that when coumarin is dissolved in alkali, the initial product is the non-fluorescing *cis*-form of *o*-hydroxycinnamic acid, which is converted by irradiation into an isomeric fluorescing *trans*-form (Feigl, 1955).



It was found that some coumarins derived from umbelliferone could not be differentiated from some flavonoids which give blue or green fluorescent under UV light (Goodwin & Kawanagh, 1950).

#### Discussion on Structure Elucidation

##### Co<sub>1</sub>

From this study, colourless long rod crystals Co<sub>1</sub> was isolated. Mass spectrum of Co<sub>1</sub> showed the molecular ion at  $m/z = 146$  corresponding to the formula C<sub>9</sub>H<sub>6</sub>O<sub>2</sub>. The mass fragmentation of Co<sub>1</sub> as shown in Figure 8.

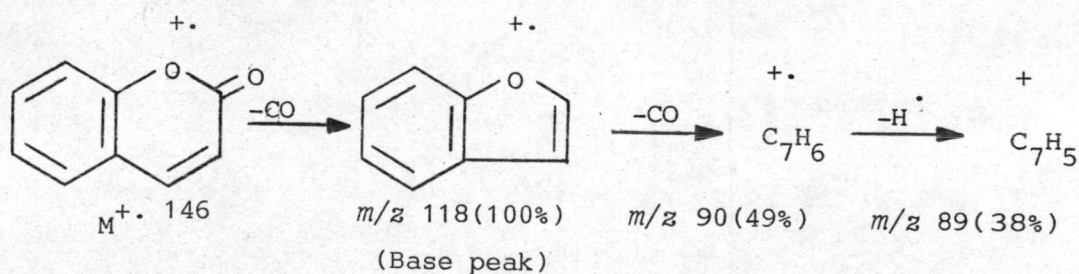


Figure 8 Mass fragmentation of Co<sub>1</sub>

Loss of CO from the molecular ion gives rise to the base peak ( $m/z 118$ ) which could be assigned as a benzofuran ion. The fragmentation proceeded further by subsequent losses of CO and hydrogen led to the ions at  $m/z 90$  and  $m/z 89$  respectively.

IR spectrum showed the presence of carbonyl ester at  $1709\text{ cm}^{-1}$  ether at  $1178$  and  $1120\text{ cm}^{-1}$  and disubstituted benzoid function at  $745\text{--}755\text{ cm}^{-1}$  (Figure 29, page 118).

UV maximum absorption of  $\text{Co}_1$  at  $\lambda_{\text{max}} 317\text{ nm}$  in methanol corresponding with the reported simple coumarin (Dean, 1963) (Figure 28, page 117).

$^1\text{HMR}$  spectrum of  $\text{Co}_1$  showed the presence of 4 aromatic protons coupling to each other. These protons could be assigned for 5-H, 6-H, 7-H and 8-H protons of simple coumarin skeleton. A pair of doublets centered at  $\delta 6.42$  and  $7.71\text{ ppm}$  (Figure 30, page 119) were assigned as the *ortho* coupling of 3-H and 4-H protons. The integration of  $^1\text{HMR}$  spectrum of  $\text{Co}_1$  suggested that there were only 6 aromatic protons which agreed with the structural formula of simple coumarin. Thus the proton chemical shifts could be assigned to the structure of  $\text{Co}_1$  as shown Figure 9.

$\text{Co}_1$  is identical in melting point, molecular weight (The Merck Index, 1983) and  $^1\text{HMR}$  spectra with those of identified coumarin spectral data (Abraham & Loftus, 1980). It is therefore concluded that  $\text{Co}_1$  is coumarin.

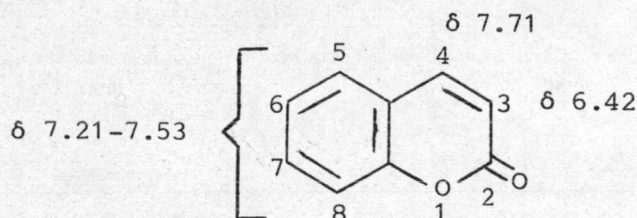


Figure 9 Proton chemical shift assignments of  $\text{Co}_1$

fl<sub>1</sub>

fl<sub>1</sub> gave positive reaction with Shinoda's reagent (magnesium and hydrochloric acid), rather weak orange solution. This test showed that fl<sub>1</sub> could be flavone (Dean, 1963). fl<sub>1</sub> was subsequently identified as a known flavone called Eupalestin.

fl<sub>1</sub> gave maximum UV absorption at  $\lambda_{\text{max}}$  334 nm in methanol (Figure 32, page 121) which is characteristic of flavone (Ngo-Li Van & Thi Van Cuong Pham, 1979). There were no change in the UV spectrum when aluminium chloride, sodium hydroxide, sodium acetate and boric acid were added in the methanolic solution of fl<sub>1</sub>. The result showed that fl<sub>1</sub> did not possess any free hydroxyl group.

IR spectrum showed the presence of a carbonyl group at  $1630 \text{ cm}^{-1}$ , ether at  $1040 \text{ cm}^{-1}$  and  $1115 \text{ cm}^{-1}$ , and aromatic HC=CH at  $1590 \text{ cm}^{-1}$  (Figure 33, page 122).

Mass spectrum of fl<sub>1</sub> showed the molecular ion at  $m/z$  416 (Figure 37, page 126) corresponding to the formula  $\text{C}_{21}\text{H}_{20}\text{O}_9$ . The mass fragmentation of fl<sub>1</sub> showed the presence of important ions at  $m/z$  241, 240, 200, 176 as shown in Figure 10, page 81.

<sup>1</sup>H NMR spectrum of fl<sub>1</sub> (Figure 34-36, page 123-125) indicated the presence of five methoxyl groups, three aromatic protons and one methylene dioxy group. The corresponding <sup>1</sup>H resonance appeared at  $\delta$  4.105, 4.016, 3.987, 3.952, 3.949, 7.149, 7.103, 6.568 and 6.088 ppm respectively. The complete proton chemical shifts could be assigned to the protons in the molecule by comparison with those of Eupalestin (Ngo Li-Van & Thi Van Cuong Pham, 1979) as shown in Figure 11, page 81.

The mass spectrum (Markham, 1982), the melting point and all other spectral data were in full agreement with those of reported values of Eupalestin (Ngo Li-Van & Thi Van Cuong Pham, 1979). It could be concluded that fl<sub>1</sub> is Eupalestin.

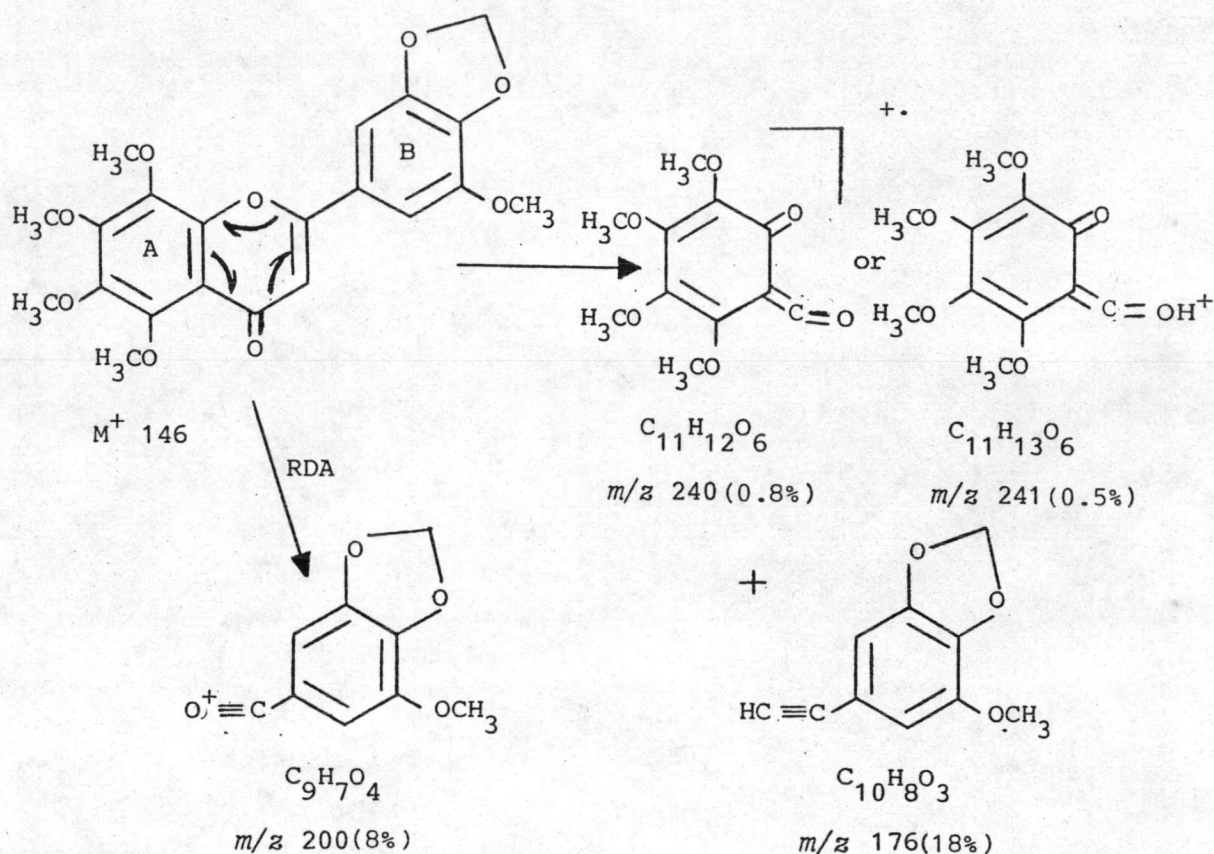


Figure 10 Mass fragmentation of fl<sub>1</sub>

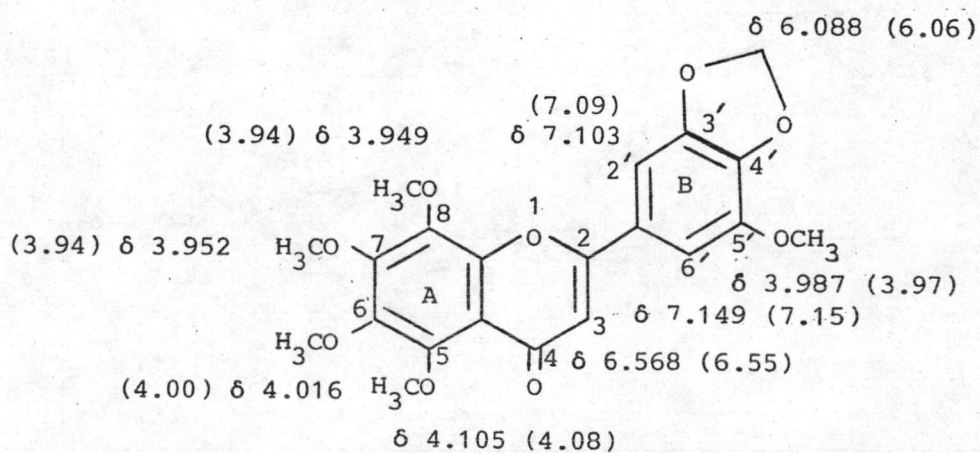


Figure 11 Proton chemical shift assignments of fl<sub>1</sub>

Chemical shift values in parenthesis are for reported values of Eupalestin (Ngo li-Van & Thi Van Cuong Pham, 1979).

fl<sub>2</sub>

fl<sub>2</sub> with Shinoda's reagent (magnesium and hydrochloric acid) gave rather weak red solution. This test showed that fl<sub>2</sub> could be flavone (Dean, 1963). fl<sub>2</sub> was subsequently identified as a known flavone called 5'-methoxynobiletin.

fl<sub>2</sub> gave maximum UV absorption at  $\lambda_{\text{max}}$  327-28 nm (Figure 38, page 127) in methanol. There were no change in the UV spectrum when aluminium chloride, sodium hydroxide, sodium acetate and boric acid were added to methanolic solution of fl<sub>2</sub>. The result showed that fl<sub>2</sub> did not possess any free hydroxyl group.

IR spectrum showed the presence of a carbonyl group at 1643 cm<sup>-1</sup>, ether at 1130 cm<sup>-1</sup> and aromatic HC=CH at 1598 and 1510 cm<sup>-1</sup> (Figure 39, page 128).

Mass spectrum of fl<sub>2</sub> showed the molecular ion at *m/z* 432 (Figure 41, page 130) corresponding to the formula C<sub>22</sub>H<sub>24</sub>O<sub>9</sub>. The mass fragmentation of fl<sub>2</sub> showed the presence of important ions at *m/z* 240, 241, 195, 192 as shown in Figure 12, page 83).

<sup>1</sup>H NMR spectrum of fl<sub>2</sub> (Figure 40, page 129) indicated the presence of seven methoxyl groups and three aromatic protons. The corresponding <sup>1</sup>H resonance appeared at  $\delta$  4.110, 4.028, 3.958 (4-OCH<sub>3</sub>), 3.932, 7.168 (2-H) and 6.639 ppm respectively. The complete proton chemical shifts could be assigned to the proton in the molecule by comparison with those of 5'-methoxynobiletin (Ngo Li-Van & Thi Van Cuong Pham, 1979) as shown in Figure 13, page 84.

The mass spectrum (Markham, 1982), the melting point and all other spectral data were in full agreement with those of reported values of 5'-methoxynobiletin (Ngo Li-Van & Thi Van Cuong Pham, 1979). It could be concluded that fl<sub>2</sub> is 5'-methoxynobiletin.

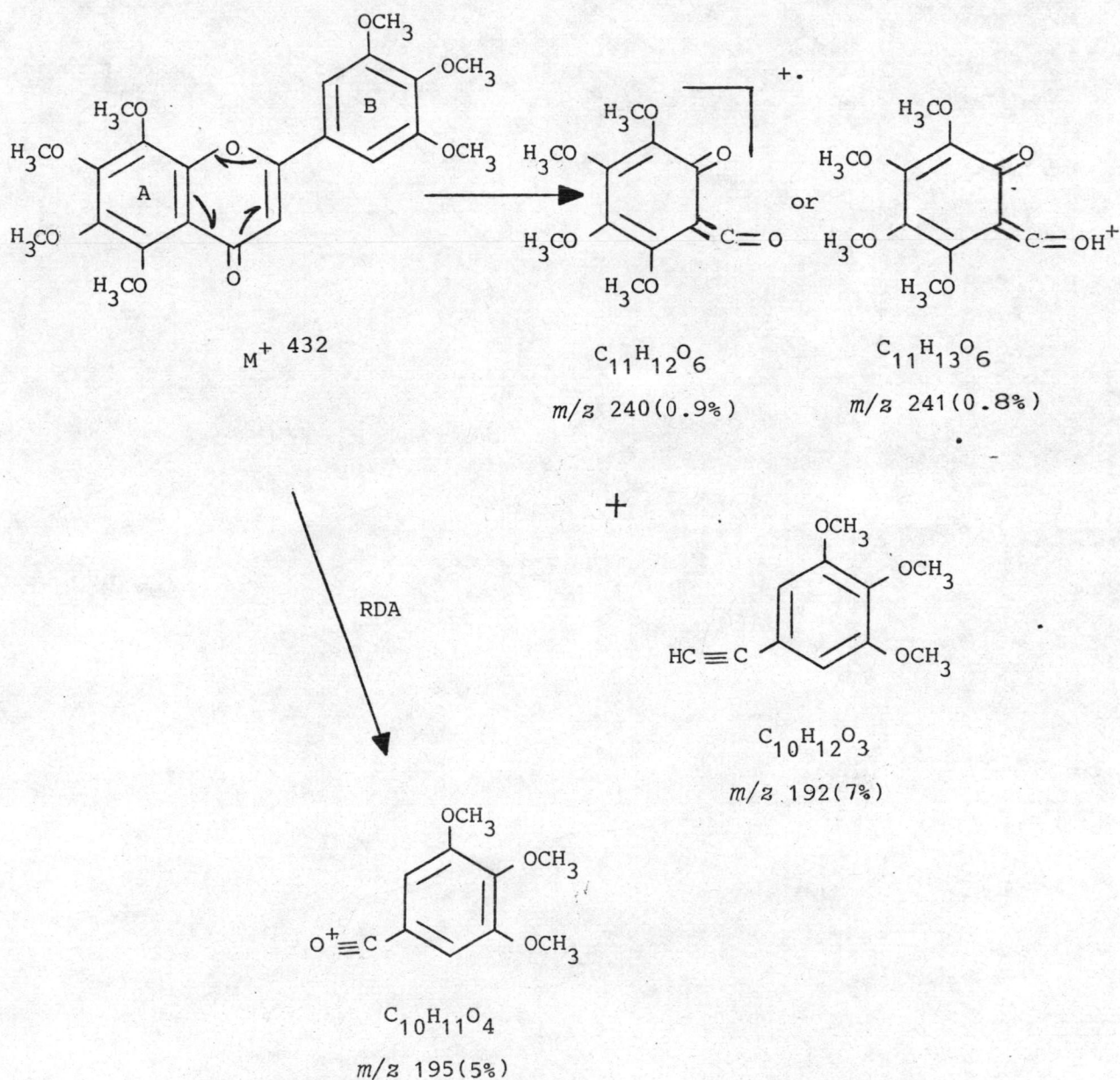


Figure 12 Mass fragmentation of fl<sub>2</sub>

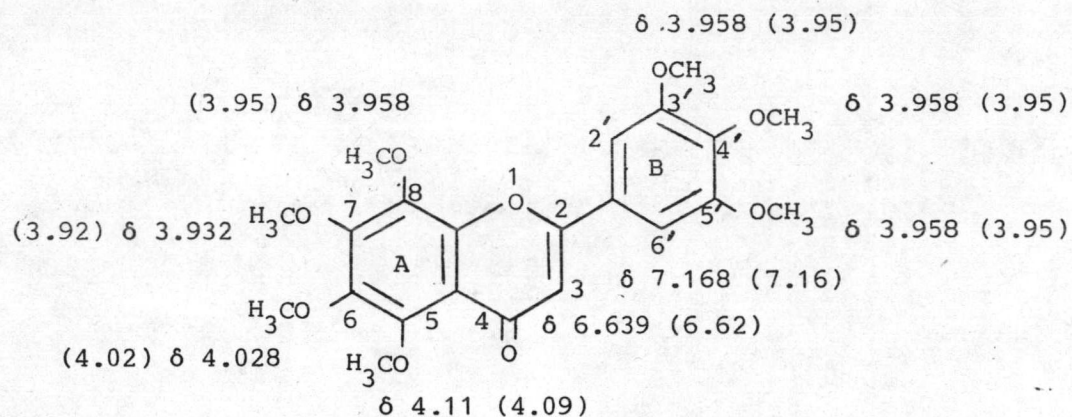


Figure 13 Proton chemical shift assignments of fl<sub>2</sub>.

Chemical shift values in parenthesis are for reported values of 5'-Methoxynobiletin (Ngo Li-Van & Thi Van Cuong Pham, 1979).

#### Discussion on Chemotaxonomy

Eupalestin was reported to be found in *Eupatorium coelestinum* Linn. (Ngo Li-Van & Thi Van Cuong Pham, 1979) and *Ageratum houstonianum* Mill. (Quijano *et al.*, 1982), but it had been never found in *Ageratum conyzoides* Linn. According to this investigation, eupalestin has been isolated from *A. conyzoides* leaves.

From the chemotaxonomic point of view, the co-occurrence of eupalestin in both *Ageratum* and *Eupatorium* is supporting the principle of classification that the two genera are arranged in the Tribe Eupatorieae.