

CHAPTER IV

DISCUSSION

1. Rutaceae and related families

The family Rutaceae comprises about one hundred and fifty genera with sixteen hundred species (Pakrashi and Bhattacharyya, 1965). Engler and Prantl divide the Rutaceae into seven subfamilies-the Rutoideae, Toddalioideae, Aurantioideae, Flindersioideae, Dictyolomatoideae, Spathelioideae and Rhabdodendroideae. Of these, the last three are monogeneric. The bulk of the genera and species in the family are found in the Rutoideae, Toddalioideae and Aurantioideae; Flindersioideae consists of two genera only (Engler and Prantl, 1931).

According to Willis, (followed the system of Engler and Prantl) the groups of which the family is made up differ considerably among themselves and several of them were formerly regarded as independent families. They are considered to be closely allied to the Meliaceae, Burseraceae, Simarubaceae, Zygophyllaceae and Cneoraceae. These relationships are set out in Fig. 9, p. 37 (Willis, 1966).

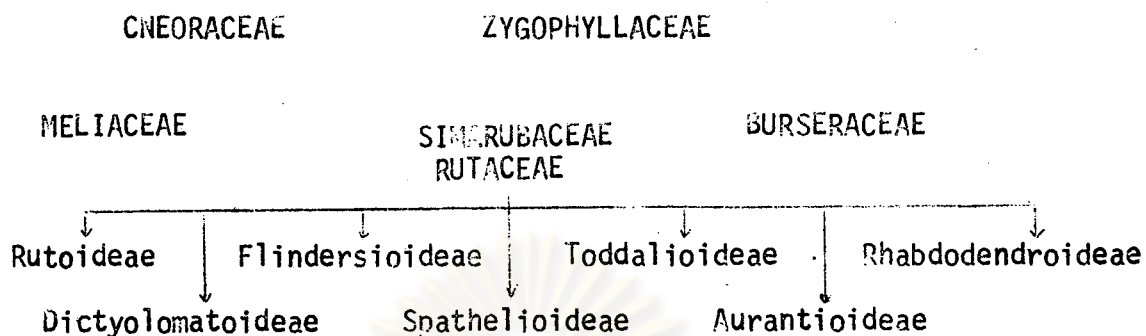


Figure 9. Rutaceae and related families.

2. Chemotaxonomic significance of alkaloids in Rutaceae

From a purely chemical point of view the Rutaceae is a fascinating group and in respect of the alkaloids it produces is probably the most versatile of all the families of higher plants. Alkaloids of diverse structural types, viz, quinoline, furanoquinoline, acridine, quinazoline, protoberberine, 1,2-benzophenanthridine, aporphine, protopine, canthinone, imidazole, pyrrolidine, oxazole and β -phenylethylamine bases, have been encountered in Rutaceae. Some of those types, viz, furanoquinoline and acridine, are restricted only to and thus diagnostic of this family. Like acridines, with a few exceptions, quinolines and quinazolines have also been found to be associated with furanoquinolines though the reverse is not true (Pakrashi and Bhattacharyya, 1965).

Among the subfamilies of Rutaceae, no information is available concerning the occurrence of alkaloids in Spathelioideae or Rhabdodendroideae. Of the four important subfamilies, Rutoideae and Toddalioideae are almost equally versatile in producing alkaloids. Aurantioideae is the only known source of simple quinazolines. Quinolines and

furanoquinolines are found throughout four of the five subfamilies, Dictyolomatoideae is the one exception. Acridines and benzylisoquinolines have been isolated from three of these subfamilies (Rutoideae, Toddalioideae and Aurantioideae) (Price, 1963).

3. Chemotaxonomic significance of coumarins in Rutaceae

In addition to alkaloids and essential oils, the Rutaceae is particularly rich in coumarins which are distributed throughout the four subfamilies Aurantioideae, Rutoideae, Toddalioideae and Flindersioideae. There is no significant variation in the overall distribution of coumarin types between the three major subfamilies. The total number of isolation of all coumarins have been made in the approximate ratio of 6:1:3 between Rutoideae, Toddalioideae and Aurantioideae. A breakdown of the isolations of linear, angular and dihydro furano- and pyranocoumarins shows little deviation from this ratio. Similarly among simple coumarins the overall ratio remains about the same although it should be noted that if the C-8 prenyl and C-3 prenyl subgroups are considered separately it swings more toward the Aurantioideae and Rutoideae (tribe Ruteae) respectively (Gray and Waterman, 1978).

Few rutaceous genera that have been thoroughly investigated have been found to lack coumarins. One group which may be atypical in this respect are the African Toddalioideae, other than *Toddalia* itself, which, despite considerable investigation, have yet to yield coumarins. The chemically atypical *Phellodendron* Rupr. (Toddalioideae) also appears to be devoid of coumarins (Gray and Waterman, 1978).

Some trends do appear to exist in the substitution patterns of coumarins. Oxygenation at more than two positions in the coumarin nucleus is relatively rare. Monosubstituted furanocoumarins occur from relatively little prenylation in the Rutoideae (Zanthoxyleae) to regular prenylation in the Aurantioideae, with the Toddalioidae and Rutoideae (Ruteae) in an intermediate position. Prenyl substitution at C-8 would appear to occur much less frequently than at C-6 and may reflect a relative rarity of the C-8 enzyme which could be taxonomically valuable (Gray and Waterman, 1978).

4. The taxonomic position of Flindersioideae

Among those families usually considered to make up the natural order Rurales (Rutaceae, Simarubaceae, Meliaceae, Burseraceae, Cneoraceae and, perhaps, Anacardiaceae) coumarins have been found only in the Rutaceae and Meliaceae. In the latter, coumarins appear largely restricted in distribution to genera of the subfamily Cedreloideae which Hartley (quoted by Gray and Waterman) has suggested may have significant morphological similarities to the Flindersioideae. Whilst the concurrence of coumarins in these subfamilies may well be pertinent to an understanding of the phylogeny of Rutaceae and Meliaceae, it must be observed that the secondary metabolism of the Flindersioideae is typically rutaceous (Gray and Waterman, 1978), for example, several *Flindersia* species have yielded the flavanone glycoside hesperidin, a characteristic metabolite of *Citrus* species, also reported from *Zanthoxylum* but not from outside the Rutaceae. Moreover, the one *Chloroxylon* species and thirteen of

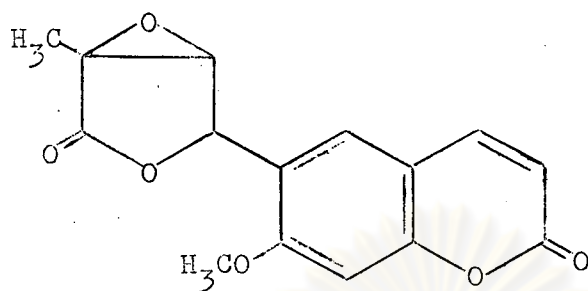
a total of fourteen *Flindersia* species contain furanoquinoline alkaloids. It will be recalled that not only have furanoquinolines not been isolated from any source other than the Rutaceae, but there is as yet not one definite identification of an alkaloid from the Meliaceae. Price considered that the Flindersioideae, Aurantioideae, Toddalioidae and Rutoideae, represented a homogeneous group distinct from the Meliaceae and other associated families (Price, 1963). However, Hutchinson placed the Flindersioideae in the family Meliaceae (Hutchinson, 1959). Other authorities agreed that it was out of place in the Meliaceae but Dadswell (quoted by Metcalfe and Chalk) suggested that it was not typical of either the Rutaceae or the Meliaceae and favoured a separate family, the Flindersiaceae (Metcalfe and Chalk, 1950).

The alkaloid flindersine has previously been reported from *Flindersia* (Flindersioideae) (Matthes and Schreiber, 1915; Brown *et al.*, 1954), *Haplophyllum* (Rutoideae) (Lavie *et al.*, 1968), *Geijera* (Rutoideae) (Dreyer and Lee, 1972) and *Atalantia* (Aurantioideae) (Bowen and Lewis, 1978). The present work is the second report of the occurrence of a pyranoquinolone in the Aurantioideae. It is of chemotaxonomic interest because Waterman (quoted by Bowen and Lewis) has suggested a possible biochemical relationship between Rutoideae and Aurantioideae. Both subfamilies may have evolved from an ancestral Rutoideae/Toddalioidae complex, and Flindersioideae may also have evolved from this same complex (Bowen and Lewis, 1978). In addition, the presence of flindersine in Flindersioideae and at least two genera of Aurantioideae has served to maintain a link between these two subfamilies. It is one of the significant means for supporting the proposal that the subfamily flindersioideae had better be placed in the family Rutaceae.

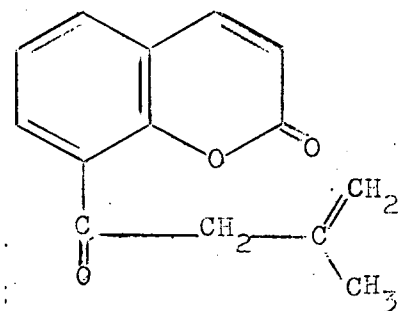
5. Biogenetic relationship between *Micromelum* coumarins and alkaloid(s)

According to the previous reports, coumarin compounds namely micromelin (micromelumin) (Lamberton *et al.*, 1967; Chatterjee *et al.*, 1968), micropubescin (Chatterjee *et al.*, 1968), microminutin (Ruangrunsi, interview 1980), 6-(2,3-dihydroxy-3-methylbutyl)-7-methoxycoumarin (Joshi *et al.*, 1975) and osthol (Price, 1963) were isolated from *Micromelum minutum* Seem. (*M. pubescens* Blume). Inspection of their structures (Fig. 10, p 42) all of these compounds are 7-methoxycoumarin derivatives. Consideration of their biogenetic pathways, C-prenylation was involved in each route, followed by further modification that might then take place on the prenyl side chain such as epoxidation, hydroxylation and intramolecular condensation (Gray and Waterman, 1978). Prenylation has been demonstrated to occur at the umbelliferone stage, other substituents being introduced later (Floss and Paikert, 1969). Consequently, the precursor of such coumarin compounds would rather be the 7-hydroxycoumarin (umbelliferone) than the 7-methoxycoumarin (hirniarin) itself. The failure of hirniarin to be prenylated indicated that a free hydroxy group is necessary on C-7 of the coumarin (Ellis and Brown, 1974).

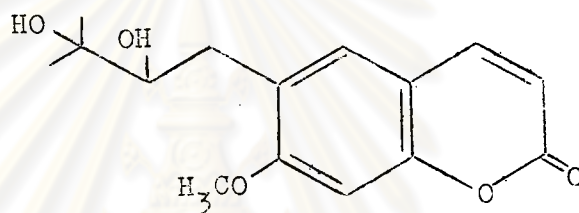
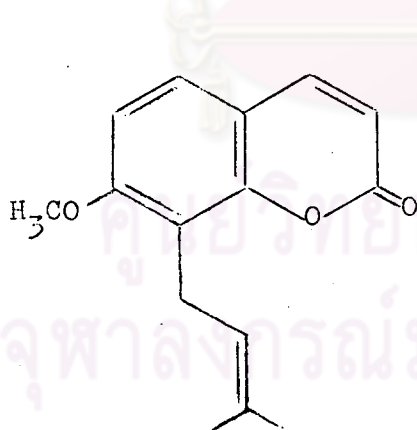
The mechanism of prenylation of umbelliferone is visualized as involving the formation of the stable anion (XIVa-c Fig. 11 p 43) which will permit the electrophilic attack of a prenyl carbonium ion at either C-6 or C-8 to yield C-prenyl coumarins or on the phenoxide to give O-prenyl compounds. Perhaps the role of the prenylating enzyme(s) is to localize the charge on the anion and thereby direct the attack of the prenyl unit (Gray and Waterman, 1978). The participating enzyme,



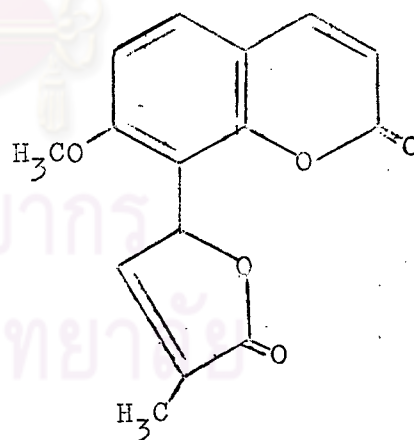
Micromelin



Micropubescin

6-(2,3-Dihydroxy-3-methylbutyl)
-7-methoxycoumarin

Osthol



Microminutin

Figure 10. Structures of coumarins from *Micromelum minutum* Seem.
(*M. pubescens* Blume)

a particulate dimethylallyl (prenyl) transferase, was demonstrated in extracts of *Ruta graveolens* Linn. cell cultures (Ellis and Brown, 1974), as well as in fresh leaves of the same species. It converted umbelliferone to 6-dimethylallylumbelliferone, but not to 8-dimethylallylumbelliferone (Brown, 1979). The work of Grundon and co-workers has provided experimental evidence for indicating that 2,4-dihydroxyquinoline is the analogue of umbelliferone (Steck and Brown, 1970). It is possible that one or more of the same enzyme(s) could be responsible for the build up of isoprenyl side chains of both *Micromelum* coumarins and alkaloid(s).

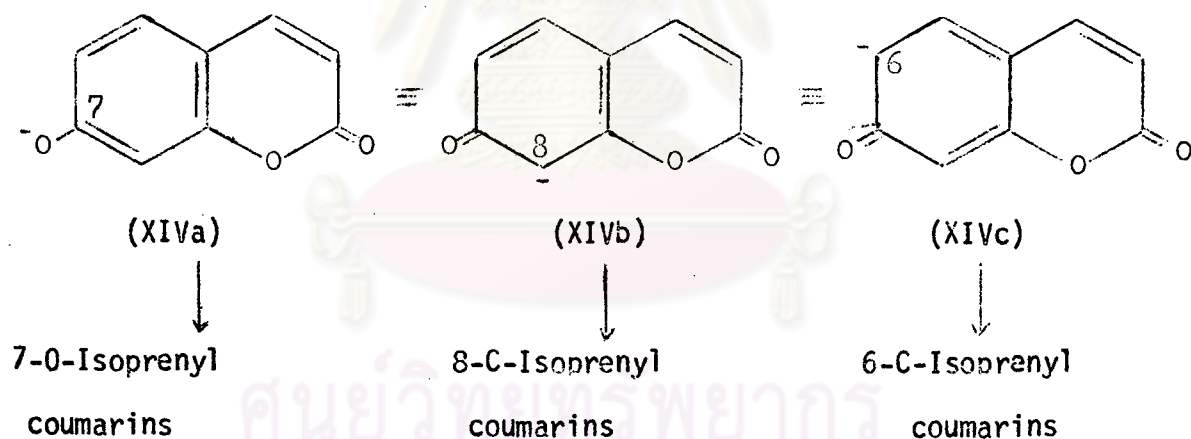


Figure 11. The mechanism of prenylation of umbelliferone.