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Chemosystematics of the *Rutaceae*: comments on the interpretation of DA SILVA & al.

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Abstract: The revision of the *Rutaceae* into 17 provisional tribes, based primarily on the distribution of secondary metabolites (DA SILVA & al. 1988) is critically reviewed. In three areas where sufficient phytochemical data is available, i.e. the "proto-*Rutaceae*", (provisional tribes *Zanthoxylum, Phellodendron, Toddalia*, and *Euodia* pro parte), the African *Toddalioideae* sensu ENGLER, (provisional tribes *Euodia* pro parte, *Acronychia*), and *Clauseneae* sensu SWINGLE, (provisional tribes *Clausena, Glycosmis, Micromelum, Merrillia*), it is shown that the proposals made by DA SILVA & al. are seriously flawed. It is suggested that for other areas of the family insufficient phytochemical information is available to justify these proposals. In a wider context it is suggested that this approach, based on only one set of characters and on a wholly insufficient data base, is unhelpful to the task of producing a new classification of the family.

In a recent paper DA SILVA & al. (1988) reviewed the secondary metabolites (primarily alkaloids, coumarins, and limonoids) of the *Rutaceae*. On the basis of their interpretation of distribution patterns and level of advancement of individual compounds (based primarily on levels of oxidation within each group – GOTTLIEB 1982), together with some morphological information (largely gleaned from ENGLER 1896, 1931), they made far-reaching proposals for the taxonomic revision of the family. In developing their arguments the authors made repeated citations of my previous papers on the chemosystematics of this family (FISH & WATERMAN 1973, GRAY & WATERMAN 1978, WATERMAN 1975, WATERMAN & GRUNDON 1983, WATERMAN & KHALID 1981).

While I do not argue with the thesis that the *Rutaceae* is in need of a fundamental taxonomic reassessment and that the distribution of secondary metabolites can play an important part in this, I view the proposals put forward by DA SILVA & al. (1988) with considerable concern. There are several reasons for this.

First, they have "reconstructed" the family into 17 provisional tribes which are almost wholly based on presently recognized taxa (usually genera used by ENGLER 1931). Unfortunately in some important cases (e.g., *Euodia, Murraya* – see below) these taxa may themselves be due for major revision. As a consequence of this their proposals provide a seriously flawed framework on which to base future discussion.

Second, all their decisions are made on the basis of incomplete phytochemical data. Few of the taxa used to compile the data used by DA SILVA & al. (1988) have been adequately examined. The diversity of secondary metabolites that the *Rutaceae* produces makes it a fascinating subject for chemotaxonomic speculation; but this complexity and novelty does have drawbacks in that, as a consequence, the family has attracted the attention of many "specialist" chemists. Such specialists are perhaps interested in only one of the several groups of metabolites that are present and will rarely identify all of the potentially systematic important classes of compounds present in the plant they are studying (e.g., for consequences see *Pentaceras, Tetradium, Haplophyllum* – below). What DA SILVA & al. (1988) have attempted to do is to formally redefine the taxonomic framework of the *Rutaceae* on the basis of information that is fragmentary for any species and which exists at all for perhaps 30% of the species of the family!

Third, the rigid methods used by DA SILVA & al. (1988) to assess the taxonomic implications of given compounds and types of compounds (from GOTTLIEB 1982) are simply not robust enough to cope with the occurrence of several of the types of compounds under consideration. For example, expression of coumarin prenylation patterns, furoquinoline and acridone oxygenation patterns, development of the acridone and carbazole nuclei, are all known to occur in taxa with little or no immediate obvious affinity (Table 1). Thus, all are likely to be polyphyletic within the *Rutaceae* or *Rutales*. Because of this and the very incomplete nature of the data, the various indices of advancement DA SILVA & al. (1988) compute for ENGLERIAN genera, and then employed to support their arguments, must be treated in a very cautionary manner.

Finally, and perhaps obviously, I am very concerned by what I regard as an overemphasis of chemical data in an attempt to contribute to the very necessary

Table 1. Some examples of probable "polyphyletic" occurrence among the secondary metabolites of the *Rutales* (all taken from WATERMAN & GRUNDON 1983 unless otherwise stated)

Metabolite	Sources
Carbazole alkaloids	Common in *Clauseneae* – *Rutaceae*, also from *Ekebergia* – *Meliaceae* (LONTSI & al. 1985)
Benzophenanthridine alkaloids	"proto-*Rutaceae*" group and *Xylocarpus* – *Meliaceae*
Quinolone alkaloids	Common in *Rutaceae*, also in *Ailanthus* – *Simaroubaceae*
8-prenylcoumarins	Concentrated in *Murraya* and allied genera (*Citroideae*) and in *Phebalium* (*Boronieae*)
Flindissol (protolimonoid)	In *Rutaceae* and *Burseraceae* (LIANG & al. 1988)
Prenylated chromones	In *Ptaeroxylaceae* and in *Skimmia* – *Rutaceae* (RAZDAN & al. 1987)

revision of the *Rutaceae*. Secondary metabolite expression is genetically controlled but is subject to the same phenotypic variation as any other character; in addition it suffers from the unique problem (in systematic terms) that the information available to the taxonomists for any given taxon is rarely complete and is often biased because of the specialized interest of the chemist unwittingly supplying it. I can see no justification in weighting these chemical data any more than the more traditional characters used in taxonomic science.

To illustrate the premature nature of the paper of DA SILVA & al. (1988) I will discuss some groups of taxa within the family where there has recently been chemotaxonomic and traditional taxonomic effort, usually collaborative in nature.

Examples from the subfamilies *Rutoideae* ENGLER and *Toddalioideae* ENGLER

The genera producing 1-benzyltetrahydroisoquinoline-derived alkaloids. DA SILVA & al. (1988) discussed at length the position of the ENGLERian taxonomic grouping subfam. *Rutoideae* tribe *Zanthoxyleae* (ENGLER 1931) and its relationship to some taxa placed by ENGLER in subfam. *Toddalioideae*. As others before them (HEGNAUER 1963; WATERMAN 1975, 1983; WATERMAN & KHALID 1981) they place great significance on the occurrence of 1-benzyltetrahydroisoquinoline (1-BTIQ) derived alkaloids in several genera (*Zanthoxylum*, including *Fagara*, *Toddalia*, *Phellodendron*, *Fagaropsis*). These alkaloids are widely held to be taxonomic markers for the primitive order *Annonales* and for the *Papaverales* and it is argued (WATERMAN 1983 and references therein) that their widespread presence in a few genera, notably of the rare and biosynthetically complicated benzophenanthridines (I) and protopines (II)*, indicates an affinity between the *Rutaceae* and these orders. Accordingly WATERMAN (1983) has used the term "proto-*Rutaceae*" to encompass the genera that produce these alkaloids, which he assumes have retained these secondary metabolite characters from their progenitors.

DA SILVA & al. (1988) respond to this state of affairs by ignoring subfamilies *Rutoideae* and *Toddalioideae*, an approach that is almost certainly justified, and by setting up three provisional "tribes", *Zanthoxylum*, *Toddalia*, and *Phellodendron* (including genera *Phellodendron* and *Fagaropsis*). Most of the remainder of *Rutoideae-Zanthoxyleae* sensu ENGLER was assigned to the *Evodia*-tribe (note *Euodia* is correct spelling), which was assumed to have arisen from *Zanthoxylum*-like progenitors (following WATERMAN 1983). One fact supporting this contention was old reports of undefined "berberine-like" (berberine is 1-BTIQ-derived) alkaloids in some species of *Euodia* (WATERMAN 1975).

Unfortunately DA SILVA & al. (1988) were unaware of an important paper published by HARTLEY (1981) in which it was proposed, on the basis of a study of nearly all the 200 or so species that had been placed in *Euodia* sensu ENGLER, that the majority were better placed in *Melicope* but that one group of eight taxa, distributed from the Himalayas to Japan and south to Java, were distinct and, morphologically, stood well apart from the type species of *Euodia*. For these taxa HARTLEY (1981) reinstated the genus *Tetradium*.

Recently we have carried out detailed chemical studies on two species of *Tetradium*, *T. glabrifolium* (NG & al. 1987) and *T. trichotomum* (QUADER & al. 1990).

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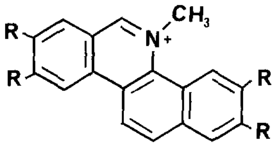
* In all formulae $R = OH$, OMe or $O-CH_2-O$.



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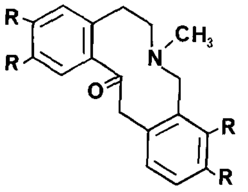

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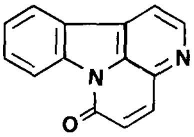

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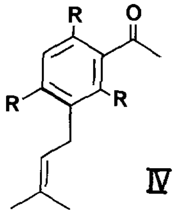

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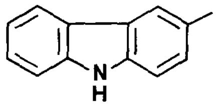

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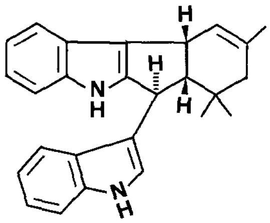

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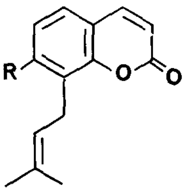

VII

From the former we isolated the benzophenanthridine alkaloid decarine as well as typically rutaceous furoquinoline and pyranoquinoline alkaloids, coumarins and limonoids. From the latter we obtained furoquinoline alkaloids, limonoids and the protopine alkaloid α-allocryptopine. These findings strongly support the proposal, made by HARTLEY (1981), that *Euodia* sensu ENGLER (as used by DA SILVA & al. 1988) is not a valid taxonomic entity; most, if not all, of the chemical features linking it with *Zanthoxylum* are now confined to species reassigned to *Tetradium*. Those species remaining in *Euodia* and in *Melicope* seem to produce little to indicate close affinity to the "proto-*Rutaceae*".

At present there are five genera which qualify for membership of the "proto-*Rutaceae*" due to the presence of 1-BTIQ-derived alkaloids. The structural types of these isolated alkaloids vary between the genera, as do the products of "modern"rutaceous metabolism (Table 2). What should we make of these differences? Two negative factors seem of particular note: (1) in numerous studies on nearly 100 different species of *Zanthoxylum* limonoids have never been reported, (2) isolation procedures used in studies on *Phellodendron* and *Fagaropsis* should have revealed other alkaloid types and coumarins if they had been present. Should we give equal importance to the absence of other classes of compounds from individual members of the group? I doubt it. This contrasts with the view of DA SILVA & al. (1988) who used the absence of alkaloids other than benzophenanthridine and quinoline derivatives from *Toddalia* as a factor supporting retention of tribal status separate from *Zanthoxylum*. It does not seem reasonable to expect a small genus like *Toddalia* (1–2 species) to match the biogenetic diversity of *Zanthoxylum* (over 200 species); what we know of the alkaloid chemistry of *Toddalia* is certainly not appreciably different from a number of species of *Zanthoxylum*. *Tetradium* is unique in being the only genus of "proto-*Rutaceae*" at present known to yield all four major classes of rutaceous metabolites (see Table 2).

HARTLEY (1981) suggests that *Zanthoxylum*, *Tetradium*, and *Phellodendron* "appear to be related to one another in a linear sequence" and that, despite differences in fruit anatomy, *Toddalia* is probably a close relative of *Zanthoxylum*, although not part of the sequence. The true position of *Fagaropsis* in this grouping needs further study but on chemical grounds a place allied to *Phellodendron* seems reasonable.

Thus morphological evidence seems to support close relationships between the "proto-*Rutaceae*" group of genera. The splitting of this group into three tribes plus part of a fourth seems unnecessary and unreasonable on present evidence. Furthermore recognition of *Tetradium* as part of the "proto-*Rutaceae*" eliminates from the *Euodia*-tribe sensu DA SILVA & al. (1988) that element suggestive of a link to the *Zanthoxylum*-tribe sensu DA SILVA & al. (1988).

Pentaceras. The monotypic genus *Pentaceras* occurs only in NE. Australia. As pointed out by DA SILVA & al. (1988) this taxon is difficult to place on morphological grounds and they assigned it to a tribe on its own. Until recently our only phytochemical knowledge of *Pentaceras* was the record of three canthin-6-one type alkaloids (III). DA SILVA & al. (1988) argue that this evidence suggests *Pentaceras*

Table 2. Distribution of major classes of secondary metabolites among the genera of the "proto-*Rutaceae*". *A* – *D* 1-BTIQ derived alkaloids: *A* aporphines, *B* berberines and tetrahydroprotoberberines, *C* benzophenanthridines, *D* protopines; *E*, *F* tryptophan-derived alkaloids: *E* canthin-6-ones, *F* indoloquinazolines; *G* alkaloids based primarily on anthranilic acid

Genus	Alkaloids							Prenylated coumarins	Limonoids
	A	B	C	D	E	F	G		
Zanthoxylum	+	+	+	+	+	+	+	+	
Toddalia			+				+	+	
Tetradium			+	+		+	+	+	+
Phellodendron	+	+							+
Fagaropsis			+						+

occupies a position between their tribes *Zanthoxylum, Phellodendron*, and *Euodia*, although on what basis they reach this conclusion is not explained. Within the *Rutaceae* canthinones are, to date, restricted to *Zanthoxylum*, although not as suggested by da Silva & al. (1988) only to Australasian species (an observation they erroneously attributed to Waterman 1975). Otherwise canthinones seem to occur quite widely in the allied family *Simaroubaceae* (Mester 1983).

However, da Silva & al. (1988) are wrong in stating that *Pentaceras* does not contain other more typically rutaceous metabolites; the truth is that they had not been looked for! In a recently completed re-investigation of the stem bark of *Pentaceras australis* carried out in our laboratory (M. A. Quader & al., unpubl.) prenylatd coumarins typical of the *Rutaceae* have been found as well an canthinones. What this means in terms of the systematics of *Pentaceras* is unclear, but it does show that the genus is capable of producing at least one group of "mainstream" modern rutaceous metabolites.

The *Acronychia*-tribe sensu da Silva & al. (1988). Another contentious proposition put forward by da Silva & al. (1988) is the linking of the SE. Asian and Australasian genera *Acronychia* and *Baurella* with a number of African genera including *Vepris, Araliopsis, Oricia*, and *Teclea*. These African taxa have been extensively studied (Dagne & al. 1988) and are a major source of furo- and pyranoquinolone and acridone alkaloids. While chemically there are some similarities, notably in the production of acridones, it is a major step to link these African and SE. Asian-Australasian genera. While none of these taxa has been found to produce coumarins some *Acronychia* do produce prenylated acetophenones (i.e. IV) which have not been isolated from any of the many African species studied (Dagne & al. 1988). Such acetophenones are also found in *Melicope* (Ng & al. 1987) and, in an important taxonomic revision not noted by da Silva & al. (1988), Hartley (1974, 1982) suggested that *Acronychia* is closely related to *Melicope* and he submerged *Baurella* in *Sarcomelicope*, a genus not mentioned by da Silva & al. (1988) and which Hartley also considers to be allied to *Melicope*.

Thus, on presently available interpretations of morphological features and on extant phytochemical data, the move to link *Acronychia* to the African genera more closely than to Australasian genera like *Melicope* seems unwarranted.

Further general comments on the *Rutoideae/Toddalioideae*

The above comments have dealt with only a small portion of the genera of the *Rutoideae/Toddalioideae* subfamilies. Among the remainder there is widespread occurrence of typically rutaceous quinoline alkaloids and coumarins and, to a lesser extent, of limonoids. At present I doubt if we have a sufficiently comprehensive understanding of the chemistry of any taxon to allow us to make definitive statements. I would be particularly cautious about reaching conclusions concerning the relative production of alkaloids and coumarins in genera like *Haplophyllum* (cf. da Silva & al. 1988). Most work on this genus has been undertaken in the U.S.S.R., and while initially the emphasis was placed on the analysis of alkaloids, there has over the last ten years been a comparable search for coumarins. So a compilation of alkaloid data for *Haplophyllum* in 1975 (Waterman 1975) revealed 34 alkaloids from 15 species and a similar compilation for coumarins (Gray & Waterman 1978) showed only 10 compounds from 5 species. By contrast in 1983 the number of alkaloids had increased to 48 from 20 species while coumarins had jumped to 36 from 15 species (GRAY 1983, MESTER 1983). Consequently a genus that 15 years ago would have been regarded as predominantly alkaloid-producing must now be considered to be a balanced producer of both alkaloids and coumarins.

An example from the *Citroideae – Clauseneae*

The *Clauseneae* sensu SWINGLE (1938) are considered to be the most primitive group in the citroid subfamily. The traditional classification (SWINGLE 1938) divides the subfamily into three tribes, *Micromelinae* (*Micromelum*), *Clauseninae* (*Glycosmis, Clausena, Murraya*) and *Merrilliinae* (*Merrillia*). DA SILVA & al. (1988) chose to emphasize phytochemical differences between genera despite the widespread occurrence of carbazole alkaloids (V) in all genera except *Merrillia*. Their conclusion was to separate this group into four provisional tribes, *Glycosmis, Micromelum, Clausena* (including *Murraya*), and *Merrillia*.

This interpretation is again confounded when one makes a more comprehensive survey of the phytochemistry. This we have recently undertaken (KONG & al. 1986a, b, 1988a, b) in a search for sources of the biologically-active alkaloid yuehchukene (VI). These investigations revealed that different taxa were characterized by the occurrence of either (a) carbazole alkaloids (V) or (b) yuehchukene and 8-prenylated coumarins (VII). The genera *Clausena* and *Glycosmis* were uniformly of type (a); while a number of *Clausena* spp. produce furocoumarins none, to date, produces significant amounts of 8-prenylated coumarins. In *Murraya* some species were of type (a) but others were of type (b). *Merrillia* was also of type (b) while *Micromelum* was unique in producing the compounds typical of both (a) and (b). The chemotaxonomic division in *Murraya* was further confirmed by an analysis of volatile oils (LI & al. 1988). This showed that species of type (b) were characterized by a predominantly sesquiterpene oil while those of type (a) were rich in monoterpenes.

This division in *Murraya* into sectt. *Murraya* (type b) and *Bergera* (type a) conforms exactly to that proposed for the genus *Chalcas* nom. ill. (= *Murraya*) by TANAKA (1929) and has been discussed in detail (BUT & al. 1988). TANAKA (1929) went further in proposing that sect. *Bergera* showed affinities to *Micromelum* while sect. *Murraya* approached *Merrillia*.

There is therefore a clear dichotomy in this group of taxa in which the presence of alkaloids derived from 3-prenylindole (such as yuehchukene), 8-prenylcoumarins, and perhaps polymethoxylated flavonoids represent one line (*Murraya* sect. *Murraya, Merrillia*) and alkaloids derived from 2-prenylindole (carbazoles) represent the other (*Murraya* sect. *Bergera, Clausena, Glycosmis*). Both lines come together in *Micromelum*, probably at the basal end of the phylogeny. Within the carbazole line many other rutaceous metabolites appear, including furocoumarins, furoquinoline and acridone alkaloids, and limonoids. It is impossible at present to attribute any taxonomic significance to these in terms of the *Clauseneae* sensu SWINGLE as no systematic search has been carried out.

The coherent chemical patterns outlined above offer a far more reasonable chemotaxonomic basis for understanding relationships in the *Clauseneae* (Fig. 1) than do the division into four tribes proposed by DA SILVA & al. (1988). The weakness of the proposal made by DA SILVA & al. (1988) stems from an unwarranted

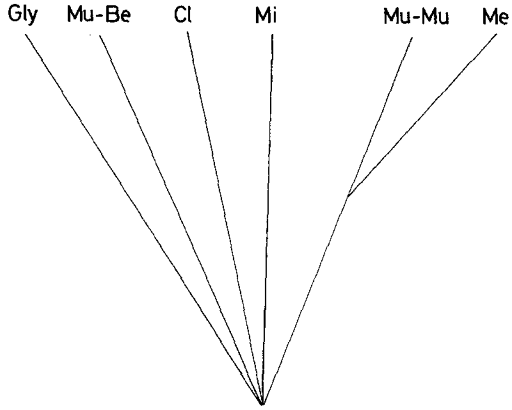

Fig. 1. Possible phylogeny for the *Clauseneae* SWINGLE based on the distribution of 2-prenylindole (carbazole) and 3-prenylindole alkaloids and 8-prenylated coumarins. Cl *Clausena*; Gly *Glycosmis*, Me *Merrillia*; Mi *Micromelum*; Mu-Be *Murraya* sect. *Bergera*; Mu-Mu *Murraya* sect. *Murraya*

acceptance of the genus *Murraya* as a discrete entity and a separation of other genera of the *Clauseneae* on the basis of insufficient phytochemical data, a problem that plagues interpretation throughout their paper.

Concluding comments

In the above examples, particularly those based on the "proto-*Rutaceae*" and *Clauseneae* sensu SWINGLE groups I have demonstrated how powerful secondary metabolite profiles can be in an analysis of the systematics of the *Rutaceae*. In both cases I have made use of extensive data that was either not available to or that was missed by DA SILVA & al. (1988). In both examples their interpretations have been shown to be seriously flawed. It is my contention that it is only in these two parts of the family, and perhaps in the African taxa of the *Toddalioideae* sensu ENGLER, that the level of chemical information available is sufficient for a detailed analysis of this type. Furthermore, in these areas the success of the chemotaxonomic approach has been due largely to the parallel and often collaborative efforts of comparative phytochemistry and traditional systematists.

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