

CHEMOSYSTEMATICS AND COEVOLUTION

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ABSTRACT

The utility of secondary chemical characters employed in plant systematics is reconsidered in the light of their ecological importance in plant-animal interactions. The taxonomic significance of biochemical characters representing adaptations to environmental stress is also considered. Taxonomic aspects of the increasing number of secondary compounds found in animals are briefly discussed. Methods of applying chemical data to systems of biological classification are reviewed.

INTRODUCTION

Chemosystematics, which is the application of chemical characters to problems of classification and phylogeny among living organisms, first developed as a distinct subdiscipline of organic chemistry some fifteen years ago. Developments in this field were first discussed in depth by IUPAC at the 4th Natural Products Symposium, held at Stockholm, Sweden in 1966, when section 4 of the programme was devoted to the topic (see ref. 1). Since then, at least two important international gatherings have been held on chemosystematics, one organized by IUPAC at Strasbourg, France in 1972 (see Swain, 2) and the second initiated by the Nobel Foundation at Lidingo, Sweden in the following year (see Bendz and Santesson, 3).

My task, in this lecture, is to attempt to summarize the progress that has been made in this field, since these last two Symposia were held.

FUNCTION AND TAXONOMIC IMPORTANCE

Perhaps the most radical development influencing chemosystematics recently has not been in this field *per se*, but in a neighbouring one, that of chemical ecology. In the early days of chemosystematics, it seemed as if the taxonomically interesting natural products were those which were functionless. As Lord Todd and others have put it, the immense diversity of secondary chemistry appeared to be produced in plants simply to keep organic chemists happy with new structural problems to solve. The value of alkaloids and complex terpenoids as systematic markers was enhanced by the view, often expressed at that time, that they were simply waste products of primary metabolism. Because they were generally very variable in their natural distribution, they appeared to be ideal for purposes of classification.

This situation has been completely changed by developments in the field of plant-animal interactions, which have led to the growing realisation that the so-called secondary compounds are of prime importance in relation to ecology. They are particularly important in defence, provide the chemical signals of pheromonal interactions, contribute towards disease resistance, are involved in allelopathy and so on. These developments have been succinctly summarized by Swain (4,5) in two reviews, entitled "Secondary compounds: primary products" and "Secondary compounds as protective agents". Recent books covering these advances in phytochemical ecology include those of Gilbert and Raven (6), van Emden (7), Harborne (8) and Sondheimer and Simeone (9). The possibility of secondary substances being involved in primary metabolism has also been cogently argued recently by Seigler and Price (10).

Because of these developments in chemical ecology, it is timely to reconsider the role of plant and animal secondary chemistry in terms of functional importance in relation to their value in taxonomy and phylogeny. This problem of the utility and adaptive value of taxonomic characters is, of course, not a new one. It was discussed with regard to biological features in a Systematics Meeting as long ago as 1959 (see Cain, 11). In the past, many taxonomists have held the view that only non-adaptive (non-functional) characters were of real importance in taxonomy. One of de Candolle's stated fundamental principles was to reject physiological characters in plants from taxonomic consideration and to base classification solely on comparative morphology. Cain (12), an animal taxonomist, was one of the first to point out that this was much too simplistic a view of the situation and that

many physiological characters could be of assistance, particularly when considering phylogenetic trends.

Cain has also indicated that the term 'trivial' or 'non-functional' character is really a misnomer and only refers to our ignorance regarding the function of a particular character, rather than that function is absent. Indeed, he has given examples where 'good' taxonomic characters, utilized because of their apparent insignificance, subsequently turned out to be of singular importance to the organism. Thus, the various shell colour patterns of snails were utilized as taxonomic characters, long before it was realised that these patterns provide valuable protection and camouflage to the creatures from bird predation. An apparently insignificant bar on the underside of a particular fly species, a character employed to distinguish it from related taxa, turned out to be of vital importance in permitting it to land upside down on ceilings!

Although protein data will not be considered in detail in this review, it is worth pointing out here that there is a similar controversy in molecular evolution whether mutations in amino acid sequences are neutral or of adaptive value. The extreme regularity of amino acid substitution in the invariant positions of the sequences of many proteins has led to suggestions that molecular evolution at variant sites is 'neutral', occurring as a randomly and adaptively unselected process at a constant rate with time (e.g. Ohta and Kimura, 13). This view has been seriously questioned in recent years (Swain, 14). Crowson (15), among others, has attacked this proposition, even in the case of such a relatively conservative protein as cytochrome *c*. Crowson points out that some invariant sequences (positions 2, 5 and 8) in the vertebrates vary in the insects, while others (7, 21, 55, 69 and 90) constant in animals vary in the angiosperms. This at least suggests that adaptive selection can occur at variant sites in the sequence. The idea that protein sequences mutate at a constant rate with time has also been shown to be incorrect. A dramatic example of differing rates of change is provided by two plant proteins of similar length (ca. 100 residues), cytochrome *c* and plastocyanin. While cytochrome *c* is almost invariant at the family level (Boulter 16) (but see the case of *Guizotia* and *Helianthus* in the Compositae, ref 17), plastocyanin can show as many as twenty amino acid differences between two closely related legumes, *Phaseolus* and *Vicia* (18).

Leaving aside the macromolecular data, it is now intended here to consider some of the secondary chemicals used as systematic markers in the light of what is known of their functional importance. Some physiological characters in plants will also be considered and it will be shown that their distribution is of taxonomic significance. A brief mention will be made of alkaloids, phenols and terpenoids in animals, since an increasing number of secondary compounds of defence have been discovered in the last decade. Finally, the application of chemical data to systematics will be reviewed and recent progress in the interpretation of chemical markers for biological purposes discussed.

SECONDARY CHEMISTRY IN PLANTS

Plant Pigments

Pigments are both numerous and widespread in the plant kingdom and among the many known carotenoids, quinones and flavonoids, there are a range of chemical characters which are immediately attractive for systematic consideration. Probably, the most significant and certainly the most notorious pigment character to have emerged from chemosystematic studies is that of the nitrogen-based betalain. Betalains are either purple (betacyanins) or yellow (betaxanthins) and differ from other types of water soluble pigment in being derived biosynthetically from the aromatic amino acid, L-DOPA. Plants containing betacyanins all belong to the same group of families, which fall into one natural order of the Angiospermae, the Centrospermae.

The problem with this character, and the reason for 'the great betalain battle' between chemists and taxonomists (see ref. 3) is that there are two families, the Caryophyllaceae and the Molluginaceae, of the Centrospermae in which betalains do not occur. The much more common purple anthocyanins are present in such plants. The chemists argue that the difference in pigmentation is highly significant at the biosynthetic level and they would like to see the Centrospermae divided into two orders and the separation of the two anthocyanin-containing families from all the rest (Mabry, 19). The taxonomists, on the other hand, say that the chemical character lacks phyletic significance and is not sufficient, on its own, to warrant reclassification. The order is still united in a range of biological features, including the presence of a special type of sieve-tube plastid in all the members (Turner and Bencke, 20).

The betalain controversy is still at issue and no resolution of the problem has appeared since the extensive debate on the topic at the Nobel Symposium. It seems to me

that two points, which have largely been ignored, are worth exploring here: (1) the comparative function of the two classes of purple pigment - anthocyanin vs. betacyanin; and (2) the pattern of distribution of other secondary chemical characters, and particularly flavonoids other than anthocyanins, within these 12 families. While work is in progress in my laboratory on this second point, as far as I know, no-one has yet investigated the question of function. Knowledge of this sort might well illuminate the present controversy. Are betacyanins more efficient than anthocyanins as flower pigments? Can insects and other pollinators recognize the difference between the two classes of pigment? While superficially they have fairly similar absorption spectra *in vitro*, there are small but significant differences. Perhaps these differences are more pronounced *in vivo*. It is possible that betalain synthesis represents a significant ecological adaptation to habitats containing a special range of animal pollinators.

Although the larger problem of the classification of the Centrospermae remains unresolved, the betalain character has proved useful in resolving problems of classification within the order. A series of papers have appeared, dealing with families or genera which doubtfully belong, on morphological and other grounds, to the group. The presence of betalain or its replacement by anthocyanin has provided the basis for inclusion or exclusion. The Stegnospermaceae, for example, has recently been removed from the order, on the basis of its lack of both betalains and of the characteristic Centrospermae P-type plastids (Goldblatt *et al.*, 21). It has been placed in the Capparales, because of chromosome data and the presence of glucosinolates in two of its members (Dahlgren, 22).

Except for their absence from the Centrospermae, anthocyanin pigments are otherwise universal in angiosperms. They represent a range of chemical variation, especially at the glycosidic level, of potential importance in taxonomic studies (Harborne, 23). Their value has sometimes been uncritically dismissed (see ref. 24) on the grounds of their genetic variability. This variability is only a major feature in cultivated plants and can easily be overemphasized. The fact that the pigment type can be rapidly changed by a single mutation confirms the critical function of anthocyanins in the angiosperms for providing a range of flower colours which are suitably attractive to animal pollinators.

A simple example of the phyletic value of anthocyanin patterns can be taken from a recent comparison of pigment structures in various advanced angiospermous groups. Characteristic anthocyanin structures found in three such families, the Umbelliferae, Solanaceae and Compositae are shown in Table 1. Comparison of pigments in the Umbelliferae and Compositae is particularly instructive, since these two specialized groups are united by a number of other chemical features, especially presence of polyacetylenes, sesquiterpene lactones and furanocoumarins (see Hegnauer, 25). In the Umbelliferae, the anthocyanidin is simple (cyanidin) but there is a unique and complex trisaccharide attached, as well as acylation (ref. 26). The complexity of the glycosidic moiety is similar to that found in other advanced herbaceous families, as in the Solanaceae (Table 1). By contrast, the Compositae have a remarkably simple pattern both at the aglycone and at the glycoside level. As has been argued elsewhere (ref. 27), this suggests that the Compositae is a very ancient family, retaining for the most part a simple anthocyanin synthesis which is otherwise found mainly in primitive, woody angiosperms. Evidence from other sources (e.g. Cronquist 28) agrees with this view about the origins of this vast family.

Chemical Defences in Plants

Secondary substances are currently widely used in plant systematics as chemical markers. Indeed, it is now rare for taxonomic revision at the lower levels of classification to be undertaken without some attempt being made to incorporate chemical data, even if this is only spot pattern data derived from two-dimensional chromatography. While some emphasis has been given to flavonoids (29) and essential oils (30), most other classes of secondary compound have been utilized. These include non-protein amino acids, cyanogens, alkaloids and the higher terpenoids. In all this, the substances used are thought of as being essentially non-functional, highly stable characters. Populational studies, which are beginning to be employed more and more in chemosystematics, have shown, however, that variations at this level are not uncommon.

The possibility that all these substances are of selective advantage variously to the plants in which they occur, particularly in terms of defence from predators, has now to be taken seriously following the work of chemical ecologists. Their taxonomic utility, and particularly their phyletic significance, has to be reviewed in this new light. Since Fraenkel (31) first proposed that secondary substances accumulate in leaves of angiosperms in order to deter herbivores, more and more experimental evidence for such a view has accumulated (see Feeny, 32). Alkaloids, for example, represent a class of complex structures which have the necessary qualifications for deterrence. Many accumulate in leaves or barks in great quantity, many are highly toxic to animals and many have unpleasant taste

properties, particularly that of bitterness. Such tastes provide warning signals to predators, so that feeding is arrested at an early stage.

Bitterness is not, however, a property confined to alkaloids and many other chemical structures are intensely bitter. This is true of sugar acetates, many diterpenoids (e.g. isodonal, columbin, isocolumbin) and triterpenoids (e.g. cucurbitacins and most saponins), sulphur compounds (e.g. glucosinolates) and even flavonoids (e.g. the flavanone naringin). If bitterness is a major feeding deterrent, then plants adopt a range of different chemical pathways in order to produce this end result. As Ehrlich and Raven (33) point out in their review of butterfly-plant coevolution, the irregular distribution in plants of secondary substances is immediately explicable in terms of this coevolution. As plants have developed one type of protection, insects have evolved the capacity to detoxify and cope with it, so that a second and third type of deterrent become necessary. As a result, a whole range of chemical defence strategies have been developed within the plant kingdom.

The bearing of these ecological considerations on chemosystematics is important and can certainly affect the interpretation placed on the distribution patterns of secondary substances. Three examples may be mentioned as being illustrative. The first refers to the replacement of one class of bitter toxin by another. In the order Rhoadales, as classically constituted, isoquinoline alkaloids are found universally in only two families, the Papaveraceae and the Fumariaceae. The other five or so families, such as the Cruciferae and Capparaceae, lack complex alkaloids and contain instead the regular presence of mustard oil glycosides or glucosinolates. This chemical dichotomy is recognised by taxonomists as being of significance and indeed, the order is now separated into two new groups the Papaverales and Capparales, as a result. This new division of the old order Rhoadales is supported by serological and morphological data. In ecological terms, this situation can be considered as the replacement of one feeding deterrent by another. While no one doubts the toxicity of isoquinoline alkaloids, evidence that glucosinolates are toxic has rarely been available. Erickson and Feeny (34) have now provided some experimental data on this point. They infiltrated the glucosinolate sinigrin into celery plants, at the same strength as it occurs in mustard, and found that caterpillars of umbellifer butterflies were killed as a result of feeding on the 'doctored' host plant. These authors argue that while glucosinolates can act as attractants to the handful of insect species which live on crucifers, they are essentially repellents to most insects and provide a barrier in these plants to the majority of herbivores.

An illustration of a distribution pattern of plant toxins not explicable without reference to the ecological situation can be seen in the genus *Acacia*. Of the many species in this enormous legume genus, there are some growing in South and Central America which act as hosts to ferocious ants. These ants live on the honeydew provided by the tree and, in return, protect the tree from its predators. The ants instantly emerge when the tree is approached by an animal and they sting the intruder. Such a tree needs no type of chemical protection and indeed, a survey of the leaves of these species for cyanogens gave negative results. Significantly, closely related *Acacia* species growing in the same geographical zone but unprotected by ants, uniformly contained cyanogens and other toxins in their leaves (35). In this case, the presence/absence of cyanogen cannot be regarded as a good taxonomic marker, because it only indicates which species are not protected by ants.

The importance of recognising variations in ecological strategies within a plant group of chemotaxonomic interest, is also shown in the case of legume trees which accumulate non-protein amino acids (e.g. canavanine, L-DOPA) or alkaloids in the seeds. These compounds are undoubtedly toxic to bruchid beetles which feed on the seed (36) and clearly provide in some *Prosopis* species immunity from beetle attack. While one species may be so protected, a second closely related species may completely lack toxin in the seeds. Instead, the tree provides a much larger number of seeds per generation, on the assumption that enough will survive beetle infestation to germinate and develop. Thus, a biological strategy of survival may parallel one based on chemical defence (37). This may explain why certain molecules are less valuable than others in taxonomic studies. For example, the guanidino amino acid canavanine is a useful seed character in the Leguminosae, since it characterises the Lotoideae from the other two subfamilies. Within the Lotoideae, however, its distribution is sporadic (38) and attempts to rationalize its pattern of occurrence have failed. This failure becomes explicable when the above ecological factors are taken into account.

PHYSIOLOGICAL CHARACTERS IN PLANTS

C₄ Photosynthesis

The idea of using characters derived from such a basic process as photosynthesis for

TABLE 1. Anthocyanin patterns in some specialised Angiosperm families.

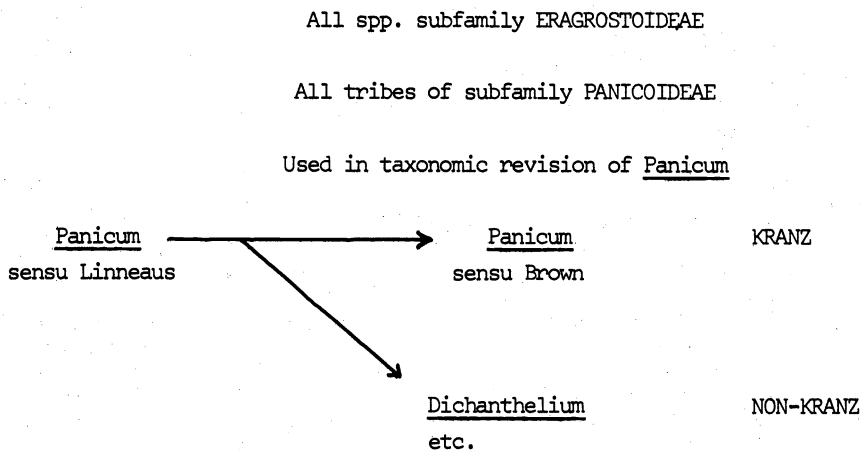
Family	Chromophore	Glycosidic pattern
Umbelliferae	Cyanidin (simple)	3-(2 ^G -xylosylgalactoside) or 3-xylosylglucosylgalactoside acylated with p-coumaric, ferulic or sinapic acid (complex)
Solanaceae	Delphinidin, petunidin and malvidin (complex)	3-rutinoside-5-glucoside acylated with p-coumaric acid (complex)
Compositae	Cyanidin or delphinidin (simple)	3-glucoside or 3,5-diglucoside (simple)

Only major patterns are shown. Other families in the Tubiflorae (e.g. Gesneriaceae, Convolvulaceae, Labiatae) are similar to Solanaceae.

TABLE 2. Plant families with Kranz Syndrome.

Aizoaceae	Gramineae
Amaranthaceae	Cyperaceae
Chenopodiaceae	—
Molluginaceae	Euphorbiaceae
Nyctaginaceae	Zygophyllaceae
Portulacaceae	—
—	Compositae

TABLE 3. Distribution of the Kranz Syndrome in Grasses.



systematic determinations would have horrified taxonomists of the Classical Era. Nevertheless an excellent example of the utilization of functional characters in taxonomy is presented by recent distributional studies of C_4 photosynthesis. As is now well known, plants with C_4 (as distinct from C_3) photosynthesis have a more efficient carbon pathway, because of the presence of an additional biochemical cycle, the so-called Hatch-Slack Pathway. This represents an adaptation to tropical climates and is restricted to a relatively small number of plant species. C_4 plants are also anatomically distinct, and the various anatomical features that differentiate such species are termed the Kranz syndrome. Depending on one's viewpoint, the two classes of plant are termed C_4 or Kranz and C_3 or non-Kranz. Indeed, both biochemical (measurement of $^{13}C/^{12}C$ ratios) and microscopic techniques can be used to determine this condition. Surveys can be carried out rapidly on small bits of herbarium tissue.

A series of papers mapping out the distribution of C_4 plants in the angiosperms have appeared during the last few years (e.g. 39 - 43). The character occurs in 11 angiosperm families (Table 2), the distribution being far from haphazard. It will be noted that no less than six of the eleven families belong to the same natural plant order, the Centrospermae, a group already distinguished from other plants by their unusual type of betalain pigmentation (see previous section). Furthermore, two of the remaining families - the Gramineae and Cyperaceae - are recognised as being closely related in many features. All families, without exception, are relatively advanced and herbaceous, so that C_4 photosynthesis is apparently a specialised feature of the angiosperms, which agrees with the more complex anatomy and biochemistry present in such plants.

The data in Table 2 indicate that the overall distribution of C_4 photosynthesis in plants is not of major taxonomic significance, since the feature is clearly polyphyletic, having arisen several times during the course of angiosperm evolution. It is in the narrower confines of the subfamily, tribe and genus that the character takes on taxonomic significance. For example, in the Gramineae, it occurs in the whole of the subfamily Eragrostoideae without exception (over 60 taxa surveyed). It also occurs regularly in the Panicoideae subfamily, being present in all tribes except for the small group Isachneae and some genera of the Paniceae. Within the Paniceae, the genera considered to be the most primitive on other grounds are nearly all non-Kranz. At the generic level in *Panicum*, the character is, in fact, of value in taxonomic revision. The Linnean concept of *Panicum* has been under fire in recent years because of its heterogeneity and many taxa have been removed to create new genera such as *Dichantherium* and *Hymenache*. The correctness of these decisions is nicely reflected in the fact that all removed species are non-Kranz, while all remaining true *Panicums* are Kranz (Table 3) (40).

Surveys for the C_4 condition in the Cyperaceae (43), Compositae (42) and the Euphorbiaceae (41) have yielded results of taxonomic value. Undoubtedly, this character, which is essentially an adaptive feature to tropical and subtropical environments, is in spite of this drawback a useful taxonomic marker. Our knowledge of the distribution of C_4 and C_3 plants is still relatively limited, but it will be clearly worthwhile extending this in the future because of the systematic potential.

Flavonoid sulphates in plants

A new group of flavonoids has recently been recognised as being of regular occurrence in plants, those with one or more hydroxyl groups conjugated with inorganic sulphate. Nearly fifty different flavone and flavonal sulphates have now been characterised as occurring in at least 200 species representing more than 20 families (44; and unpublished data). Related hydroxycinnamic acid sulphates are also found, especially in the Fluviales (45), and there is evidence that a range of other plant phenols can occur conjugated in this way.

The character seems to be ecologically important, in that it is most pronounced in water plants, especially those which occur in or near the sea. Many, but not all, halophytes characteristically contain up to 50% of their leaf flavonoid bound to sulphate. Evidence that sulphates are correlated with the halophyte condition was produced, for example, during a random survey of 80 species of Inuleae (Compositae) for their flavonoid patterns. Eight contained sulphates and a study of the collecting sites of these eight species showed that seven were obtained from either the seashore or from inland salt lakes (46). In simplest terms, synthesis of these phenolic conjugates could represent a method of absorbing excessive amounts of inorganic sulphate ion. Preliminary ecological studies, however, indicate that the process is more complex than this. The association between sulphate occurrence and water or salt stress is nevertheless reasonably clear.

In spite of these ecological relationships, surveys have indicated that the presence/absence of sulphate conjugation can be of systematic interest, just as in the case of C_4

photosynthesis. For example, in the order Parietales, sulphates occur consistently in Bixaceae, Frankeniaceae and Tamaricaceae and are present occasionally in Guttiferae and Cistaceae (Table 4). By contrast, they are absent from all species so far studied of Cochlospermaceae, Flacourtiaceae, Turneraceae and Violaceae. The taxonomic significance of this

TABLE 4. Flavonoid profiles in families of the order Parietales.

Family	Chemistry*		Biology after Cronquist
	Flavonoid sulphates	Ellagic acid	
Cistaceae	(+)	+	9
Guttiferae	(+)	(+)	-
Frankeniaceae	+	+	11
Tamaricaceae	+	+	10
Cochlospermaceae	-	+	8
Turneraceae	-	-	5
Flacourtiaceae	-	-	1
Violaceae	-	-	4
Bixaceae	+	+	8

*5 other chemical characters used with these two to produce the family cluster analysis shown in the left hand column.

distribution pattern is enhanced by the fact that it is positively correlated, with the exception of *Cochlospermum*, with the distribution of ellagic acid in these families (Table 4). These patterns of distribution correspond well with some, but not all, classificatory arrangements of these plants. The Tamaricaceae and Frankeniaceae are especially closely related in heath-like appearance and preference for arid or saline habitats, so that the discovery of sulphates uniformly in both families is satisfying. Attempts have been made to sink *Cochlospermum* and *Bixa* into the same family. The fact that they differ in several chemical features including the sulphate character, would argue against such a taxonomic decision (47).

Flavonoid sulphates also occur in several monocotyledonous families, with some taxonomic disjunction being apparent. In the Gramineae, for example, they occur in only three of the six subfamilies in Phragmitiformes (in 40% of taxa), in Eragrostioideae (in 22%) and Panicoideae (in 15%). Like the C₄ condition, they occur largely in tropical or subtropical species. Within the genus *Saccharum*, they have a distribution which is of value in drawing taxonomic conclusions regarding the origin of the cultivated forms (48). In summary, the sulphate character is an interesting new feature in the angiosperms and while its taxonomic value is limited by its probable ecological associations, it is a character worth consideration when exploring flavonoid patterns in new plant groups.

Phytoalexin response in higher plants

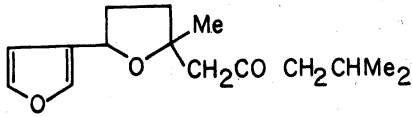
It is now well established that most higher plants respond to microbial invasion by the *de novo* production of organic compounds called phytoalexins. These compounds, although typically 'secondary' in terms of biosynthesis, are absent from healthy plants and are induced by the attacking micro-organism. Although occasionally produced in plants by other stress situations of a non-microbial nature, they are only produced consistently in plants in response to fungal invasion. Although the role of phytoalexins in disease resistance is not yet entirely clear, considerable evidence suggests that they are of importance in the protection of higher plants from fungal colonisation (49).

Although few surveys have been attempted, there is clearly a taxonomic element in phytoalexin biosynthesis, in that different plant families accumulate chemically different types of compound. Thus, the Leguminosae in general produce isoflavonoids, the Solanaceae diterpenoids, the Compositae polyacetylenes and so on (Fig. 1); anomalies are rare, for example, the furanoacetylene wyerone acid from *Vicia faba* (Leguminosae) (50). As lesser variations also occur within these families, there is the clear possibility of using phytoalexin induction as a tool in taxonomic studies. We have, in fact, applied this technique to problems of classification at the species, generic and tribal levels in the Leguminosae, with a large measure of success (51).

Phytoalexins were obtained using the elegantly simple drop-diffusate technique (Fig. 2), the non-pathogenic fungus *Helminthosporium carbonum* being used as inducer and water controls being run in every instance. After 48 hours of incubation, the phytoalexins which had diffused into the droplets were collected, isolated and identified by standard techniques. Bioassays were carried out as indicated in Fig. 3.

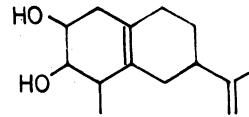
The sort of results obtainable at the generic level are shown in Fig. 4 in *Trigonella*,

CONVOLVULACEAE



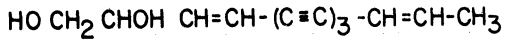
Ipomeamarone

SOLANACEAE



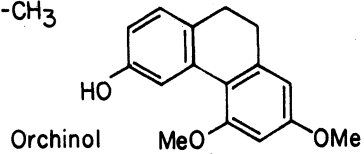
Rishitin

COMPOSITAE



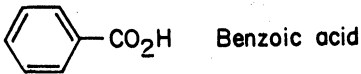
Safynol

ORCHIDACEAE



Orchinol

ROSACEAE



Benzoic acid

Fig. 1. Phytoalexins of various Plant Families

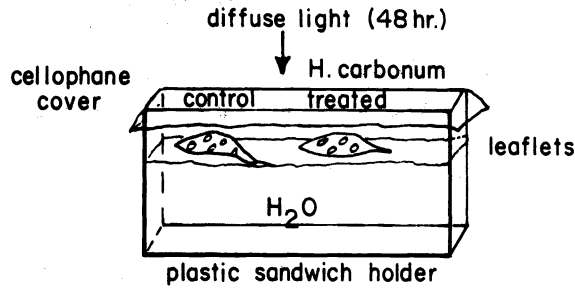


Fig. 2. Phytoalexin induction.

PHYTOALEXIN BIOASSAY

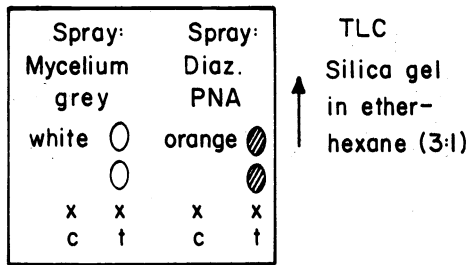


Fig. 3. Phytoalexin bioassay.

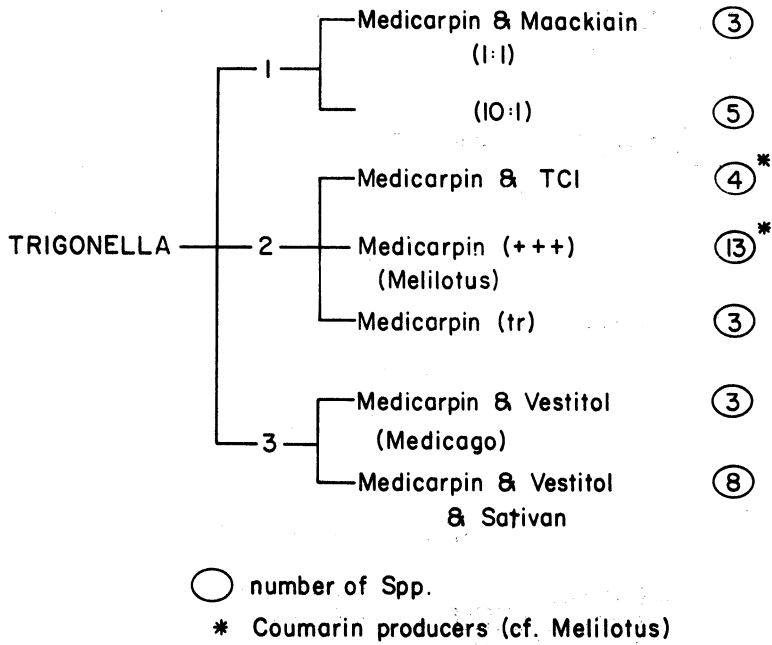


Fig. 4.

where four known compounds, the pterocarpan medicarpin and maackiain, and the isoflavans vestitol and sativan are produced (Fig. 5). In addition, a fifth phytoalexin, a new tri- or tetrahydropterocarpan (TCl) is produced in four species. The results of surveying 35

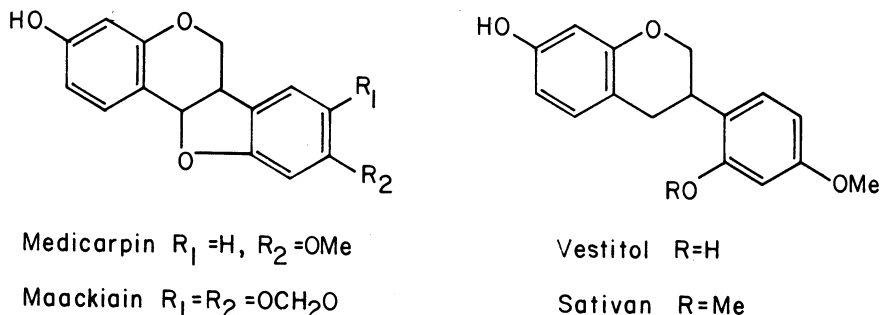


Fig. 5. Phytoalexins of *Trigonella*.

species clearly divide the genus *Trigonella* into three major groupings, with further division into minor groupings in each case. It will be noted that quantitative differences are of significance in these divisions. That these groupings are systematically meaningful is apparent from supporting morphological and chemical studies. For example, many species of group 2 show morphological resemblance to *Melilotus* a closely related genus characterised by a similar phytoalexin response. Furthermore, a special chemical feature of *Melilotus*, namely the ability to release coumarin when tissue is macerated, is present in most (11 of 16) of the species in this group (51). *Trigonella* was chosen for study because of the considerable difficulties of taxonomists in generic delimitation, so that the results from a new type of approach here could be especially valuable for systematic revision.

Similar results have been obtained in most other legume genera studied. One new type of phytoalexin, the hydroxystilbene resveratrol and its isopentenyl derivative, has been discovered in *Arachis* and *Trifolium*. Although biosynthetically somewhat distinct from isoflavonoids, hydroxystilbenes closely resemble them in structural configuration and they probably act as fungitoxins in a similar fashion. Only one genus, *Lupinus*, so far, has failed to yield a phytoalexin response; in these plants, however, preformed isopentenylisoflavones have been detected in some quantity on the leaf surface (52). Exploration of the anomalous production of wyerone acid, a polyacetylene, in *Vicia faba*, has now shown that this plant is able to produce a pterocarpan (medicarpin) in small quantity, so that isoflavonoid synthesis is truly representative of the whole family (53).

The results from phytoalexin induction are also meaningful at the tribal level and different tribes have different patterns (Fig. 6). These data have evolutionary significance, since the different types of isoflavonoid phytoalexin can be placed in a biogenetically developing series. This is shown in Fig. 7 and is based on the latest available data from biosynthetic studies (54). The efficiency of the various compounds as fungitoxins follows the same progression, isoflavones and isoflavanones being the least effective and isoflavans being the most. According to this scheme, *Cajanus* and *Lupinus* are primitive groups, members of the *Vicieae* and *Trifolieae* intermediate and *Lotus* and *Anthyllis* (*Loteae*) the most advanced. This phylogenetic scheme is not seriously at variance with ideas on tribal relationships derived from other sources (55); many critical groups, however, still need to be examined before a close correspondence between phytoalexin response and purely biological features can be assumed in the *Leguminosae* at large.

These results with the *Leguminosae* are, I believe, promising and we are using them as a basis for the systematic exploration of phytoalexin responses in other plant families.

SECONDARY CHEMISTRY IN ANIMALS

The range of structures

As a source of systematic characters, secondary chemistry has never been exploited in the animal kingdom as it has with plants. The emphasis in animals has always been on macromolecules and especially proteins. Thus, at a chemotaxonomic Symposium devoted to both plant and animals, held at Birmingham in 1967, only two papers covered such approaches in the

Tribe	Isoflavones &		Isoflavans
	Isoflavanones	Pterocarpans	
CAJANEAE	■	—	—
HEDYSAREAE	—	■	■
LOTEAE	—	—	■
TRIFOLIEAE	—	■	■
STYLOSANTHEAE	—	—	—
VICIEAE	—	■	?

Fig. 6. Phytoalexins: tribal distribution in Leguminosae.

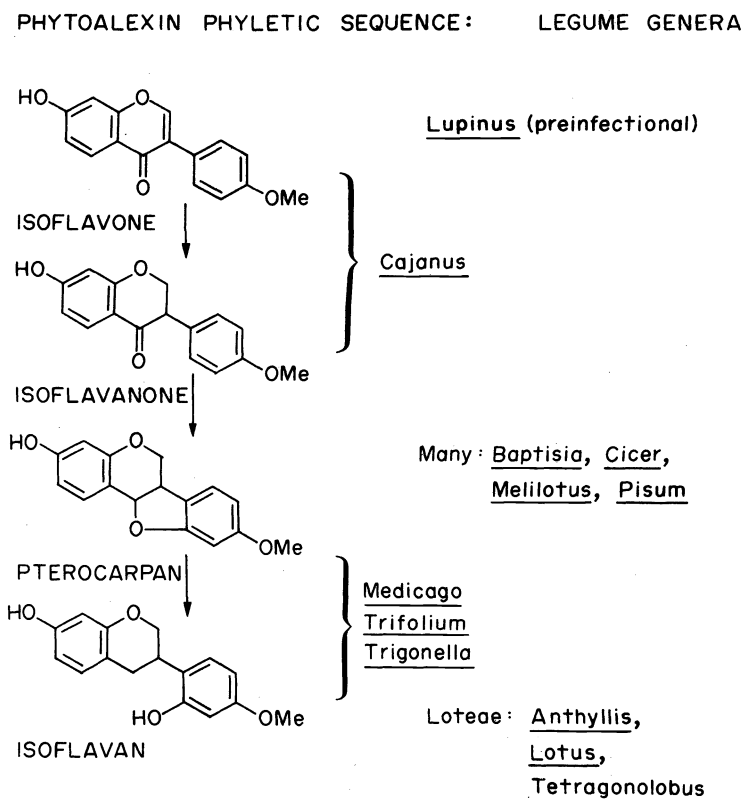


Fig. 7. Biosynthetic pathway of phytoalexins linking increasing fungitoxicity with evolutionary advancement.

animals: there was one on urinary indoles in primates (56) and another by Haslewood (57) on bile salt differences. Again, leafing through a recent comprehensive account of animal chemotaxonomy (58) one finds that any approach other than through proteins or serology receives very brief treatment.

This neglect has been partly because secondary substances are not usually thought of as being characteristically present in animals. It has also been partly because of the difficulties of obtaining sufficient animal tissue for study, particularly in the case of insects. This is probably the reason why the early discoveries of Ford (59) based on simple colour tests, that the accumulation of flavones in butterfly wings only occurs in certain taxonomic groups have never been followed up, except in one or two isolated instances (60).

The situation regarding the chemical analysis of animals has dramatically altered during the last decade, and thanks to the availability of extremely sensitive systems for separation and analysis of organic substances on the microscale, much information is available in this area of natural product chemistry. The incentives to study animal tissues for their simple molecules has largely come from interests in arthropod defence, in animal pheromones and in the economic exploitation of marine life. As a result, we know that animals are perfectly capable of synthesizing, and accumulating on occasion, a range of terpenoid, phenolic and alkaloidal substances. While some secondary compounds are undoubtedly of dietary origin, we know from feeding experiments that many others are biosynthesized *de novo* by the animal. The taxonomic implications of these new characters are only just beginning to be explored. I would like to mention briefly here recent findings with alkaloids, quinones and pheromones.

Animal alkaloids

Alkaloids, once thought to be a unique part of the richness and diversity of secondary metabolism of plants, have now been recorded in several marine organisms (61) and in a number of arthropod groups (62). While the alkaloids of butterflies, and especially those in Danainae species, are known to be of dietary origin derived by modification of ingested plant alkaloid, those occurring variously in millipedes, fire ants and ladybirds are probably synthesized by the animals themselves for defence. In the case of *de novo* synthesis, the chemistry of the alkaloids can sometimes be similar to that in plants. Witness, for example, the close structural resemblance between the alkylpiperidine toxins of fire ants and the well known alkaloid coniine of hemlock, Conium maculatum (Fig. 8). In other cases, quite new

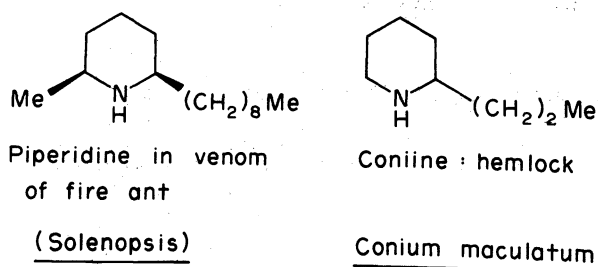


Fig. 8. Parallelism in plant and animal alkaloid synthesis.

structural types are found. This is true of the unique series of tricyclic alkaloids, found in ladybird exudates, based on the 2-methyl-perhydro-9b-azaphenalene ring system (Fig. 9).

From the taxonomic viewpoint, alkaloids of both dietary origin and of *de novo* synthesis are of interest. In the case of the Danainae butterflies, it appears that different genera modify the natural plant alkaloids ingested in different ways and one or more of three metabolites varying in the oxidation level of the exocyclic substituents may be accumulated (Fig. 10). These substances are far more than solely taxonomic characters, since they act as male pheromones and are necessary for successful mating in these insects (see ref. 62). In the case of alkaloids synthesized *de novo* such as coccinelline, six different alkaloids have been detected variously in 35 species and varieties of ladybirds. The presence of alkaloid is correlated with warning coloration and the alkaloid undoubtedly functions as a toxin to

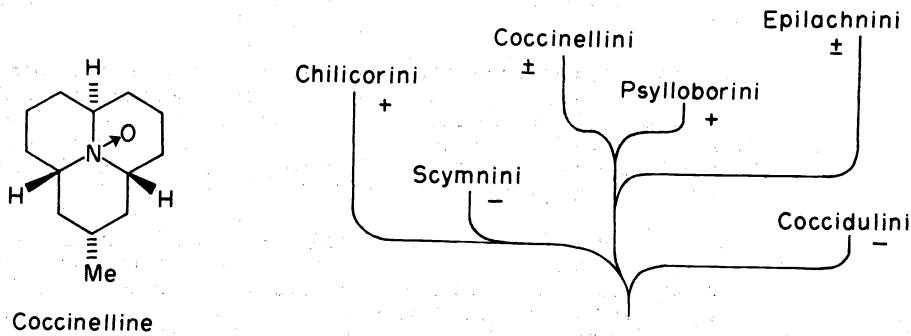


Fig. 9. Distribution of alkaloidal toxins in ladybirds (Pasteels *et al.*, 1973).

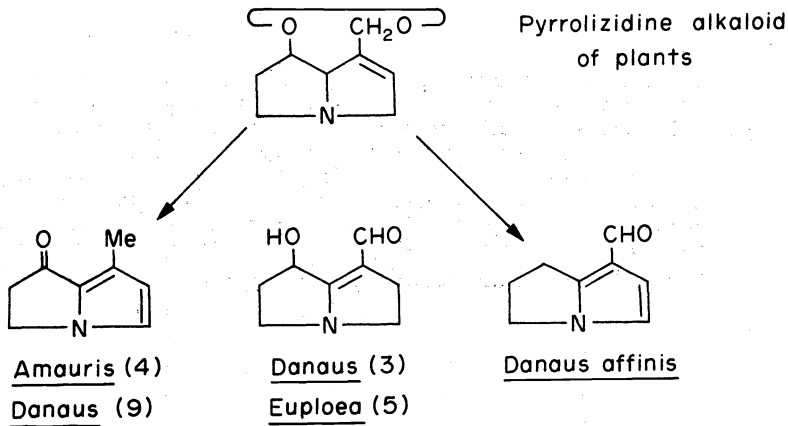


Fig. 10. Variation in wing pencil alkaloids in Danainae butterflies.

predators. Phyletically, the ability to synthesize and store alkaloid is an interesting character since it is restricted exclusively to the more advanced taxa within the Coccinellidae (Fig. 9) (63).

Animal Quinones

The best known series of animal quinones are the spinochromes, red pigments based on naphthoquinone, present in many sea urchins. Over twenty structures are known and their various occurrences in more than sixty species have been recorded by Thomson (64). However, they do not appear to have been exploited taxonomically and even their function is still largely a mystery. More recently a series of simple benzoquinones have been identified in beetles, arachnids, millipedes, earwigs and termites, where they are part of the defence secretions. In the case of black beetles (Tenebrionidae), quinones are invariably present and characterize the group. A range of quinones occur, including the simple methyl, ethyl and propyl substituents of benzoquinone. There is also 2-methyl-3-methoxybenzoquinone, and several naphthoquinones substituted in the 6-position (see Fig. 11). A recent survey of 146 species from 55 genera by Tschinkel (65) has shown that there is quite an interesting chemosystematic aspect to these defence compounds. Different quinones are significant markers at the tribal, generic and subgeneric levels (Fig. 11). Any modern taxonomic treatment of this group of beetles would have to take into consideration data from the quinone chemistry revealed by this important survey.

HANDLING OF CHEMICAL DATA FOR SYSTEMATIC PURPOSES

A major problem in the field of chemosystematics is the one of communication (see Heywood, 68). On the one hand, the natural products chemist produces new comparative data in terms of chemical structures which the taxonomist has much difficulty in comprehending or relating to the organisms involved. On the other hand, the taxonomist produces systems of classifications, which for all their many imperfections, are based on a multitude of observations derived from botanical studies made over the course of several centuries. The chemist has difficulty in recognizing both the complex biological basis and also tentative nature of most phylogenetic schemes. In this situation, it is up to the chemist to at least attempt to provide his chemical data in a form which can be understood and assimilated by the biologist. Real progress has been made recently in dealing with the problems of interpretation and I would like to mention here especially the correlation approach of Sporne, the system of scoring chemicals for biogenetic advancement advocated by Rezende and Gottlieb and the displayed phylogenetic tree of Dahlgren.

Sporne's approach to the phylogeny of the angiosperms, summarized in his recent book on angiosperm morphology (69), has been to look at correlated occurrences of those biological characters which on present day evidence can be regarded as primitive. He has found that, in the dicotyledons, no less than 24 such characters (e.g. woody habit, leaves alternate, etc.) correlate together; not only that but they also correlate with occurrence in Pre-Oligocene and Pre-Tertiary times and with presence in present-day Rain Forest floras. By mapping the number of such primitive correlated characters present in any one family, Sporne has been able to give all angiosperm families 'an advancement index' on an arbitrary scale of 0 to 100. In this system, the most primitive dicot family is Rhizophoraceae with an index of 21, the most advanced Callitrichaceae with an index of 100.

From the chemical point of view, Sporne's System is of interest because one of his primitive features is presence of proanthocyanidin, as opposed to its absence. Sporne's analyses show quite unambiguously, that proanthocyanidin, as suspected by Bate-Smith (70), is truly a primitive feature, since its occurrence is correlated with no less than 16 of the other primitive angiosperm characters. Now that such a system has been set up, it is possible to slot in other chemical characters and see whether they represent primitive or advanced features in higher plants. This has been done for presence of ellagitannin, which again is quite clearly a primitive characteristic (Fig. 12) (71). I have used Sporne's system for determining the

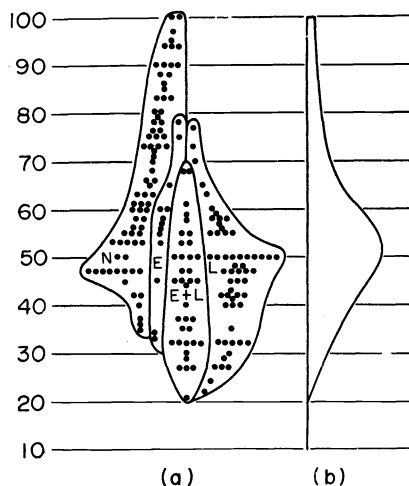


Fig. 12. Families of dicotyledons distributed vertically according to their advancement indices and horizontally according to their tannin content (L = leucoanthocyanidin, E = ellagitannin, N = no tannin). (From Sporne, 71).

relative primitiveness and advancement of a number of other flavonoid types present in angiosperms. The infrequent occurrence of biflavonoids in angiosperms, which otherwise occur profusely only in gymnosperms, can be shown to be a primitive feature on the basis of presence exclusively in families with a relatively low advancement index (average 40%) (72).

A scoring system for chemicals according to their biosynthetic complexity (and hence evolutionary advancement) was first suggested by Rezende and Gottlieb (73) for xanthenes, although the idea was developed independently for flavonoids by Bate-Smith (74). The first named authors gave all known xanthenes a particular score based on the increasing complexity in substitution pattern. Some assumptions had to be made with regard to the direction of changes in oxygenation with evolutionary advancement. The type of results, when applied to plants from which the xanthenes were obtained, is shown in Table 6. The scores shown are

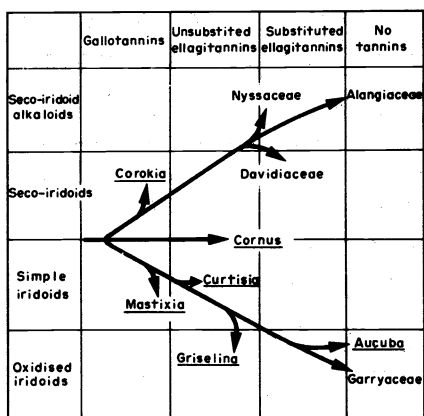


Fig. 13. Phylogenetic interrelationships in the Cornales based on phytochemical data.

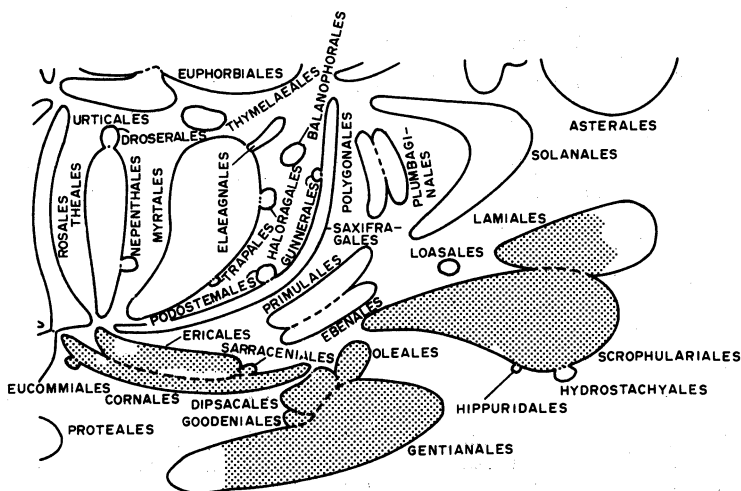


Fig. 14. Phylogenetic diagram of angiosperms showing those groups which contain iridoids

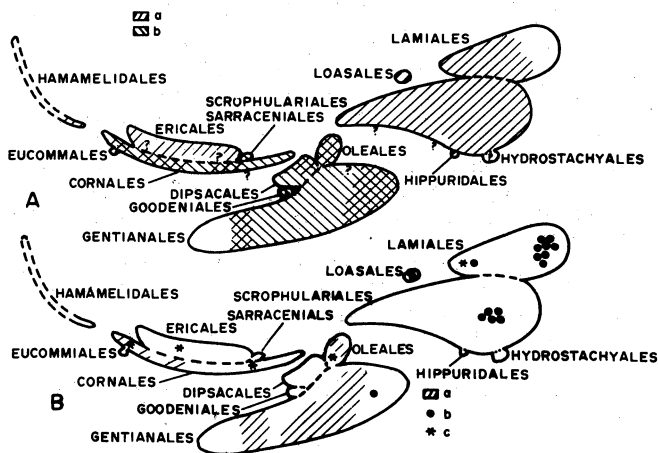


Fig. 15. Phylogenetic diagram of iridoid-containing angiosperm groups, showing the detailed distribution of different iridoid classes.

TABLE 6. Phyletic order of Gentianaceae genera.

Genus Species	No. of known xanthones	Point value
<i>Canscora</i>		2.6
<i>decussata</i>	13	2.6
<i>Gentiana</i>		3.3
<i>corymbifera</i>	1	4.0
<i>bellidifolia</i>	7	3.3
<i>lutea</i>	6	3.0
<i>kochiana</i>	4	3.0
<i>acaulis</i>	1	3.0
<i>Swertia</i>		3.0
<i>japonica</i>	6	3.0
<i>chirata</i>	3	3.0
<i>decussata</i>	3	3.0
<i>perennis</i>	1	3.0
<i>tosaensis</i>	2	3.0
<i>Frasera</i>		4.1
<i>albicaulis</i>	15	4.1
<i>carolinensis</i>	6	4.1
<i>Halenia</i>		4.7
<i>asclepidea</i>	3	4.7
<i>Macrocarpaea</i>		3.0
<i>glabra</i>	2	3.0

relatively similar and do not, perhaps, truly reflect the chemical diversity present in these plants. Such a scheme is very dependent on the thoroughness with which the different plants have been screened for their xanthones and clearly is best applied when all chemical analyses have been carried out under identical conditions. A similar scoring system has been applied to phytochemical data obtained from the family Cornaceae (75). Here the data are shown in a two dimensional scheme (Fig. 13) which displays the relative advancement of the different taxa in relation both to their iridoid and their tannin content.

Application of biochemical data to the well known phylogenetic schemes of Cronquist (76) or Takhtajan (77) is fraught with difficulty, because of the imprecise nature of these and other schemes. Boulter (16), for example, has tried to summarize his cytochrome *c* data, using such schemes as a basis, without a great deal of success. A partial solution to such problems has been devised by Dahlgren (22), who has produced a phylogenetic system of angiosperm classification, which can be used to demonstrate the distribution of any new biological or chemical feature which might arise. The application of this system to the known distribution of iridoids is particularly interesting, (Fig. 14) since it reveals that all the families that contain these substances are phyletically linked to each other. The same system can be refined to show the more detailed distribution of the different structural classes within the iridoid group (Fig. 15). All these techniques are important in exhibiting in new ways the distribution of chemical characters in plants and their application can only have a beneficial effect in increasing the understanding between chemists and systematists.

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