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Perspectives in Chemotaxonomy

Studies of secondary compounds in plants may provide knowledge of phylogenetic relationships.

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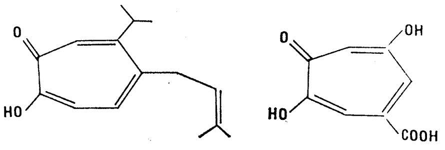
Historically, chemical taxonomy of plants developed out of the chemistry of natural products, and was limited essentially to observations of distributional correlations between groups of plants and certain of their chemical products. The possibilities of the application of chemical knowledge to the problems of systematics have intrigued both chemists and biologists for many years. The classic work of Baker and Smith (*1*) in 1920, on the oils of *Eucalyptus,* is generally cited as an example of such an application. The work of these investigators surpassed that of much later periods in that, throughout their studies, the specific oil components of *Eucalyptus* were always identified. In later work, the chemistry was often treated only superficially, but inferences of great phylogenetic significance were extracted from the meager data (*2*).

Most chemical studies devoted primarily to systematic problems in plants are concerned with groups of secondary constituents such as alkaloids, terpenes, and various water-soluble pigments. Within a particular taxonomic group the presence of certain unusual compounds may help to delimit the group (for example, the alkaloids of *Senecio* or *Veratrum*; the isothiocyanates, or mustard oils, of Cruciferae). Sometimes, distinctive chemical features provide sound bases for speculation about rather broad systematic problems. For example, the presence of isoquinoline alkaloids in a number of families of flowering plants among which evolutionary affinities had been suggested previously on other grounds provides some further support for considering such presumed relationships to be valid. On several occasions, however, it has been noted both by chemists and taxonomists that many secondary compounds are rather widely distributed among taxonomic groups which have no close evolutionary affinity to each other, and the existence of this situation makes chemotaxonomy, in their opinion, an illusion. The favorite example illustrating this point is nicotine, which is found in several widely separated angiosperm families and in the nonflowering plant, *Equisetum.* [Perhaps another alkaloid, bufotenine, should now be substituted as the stock example, since it occurs in toads and in the flowering plant, *Piptadenia falcata* (*4*).] While many such chemical evolutionary parallelisms occur in plants, as do cytological, morphological, and physiological parallelisms, they merely represent further challenges to those investigators who assume the responsibility of integrating such data with other taxonomic criteria. The presence of the chemically interesting tropolones (Fig. 1) in fungi such as *Penicillium* and in certain conifers does not lead one to infer an evolutionary connection between these two groups, but given effective methods, the study of the comparative genetic and, as a corollary, the biosynthetic and enzymatic bases of the two systems, should be of great interest. There are numerous other examples of compounds whose distributions make it difficult to determine whether the compounds are indicative of a phylogenetic connection or of independently evolved pathways. Many alkaloids, the isothiocyanates, certain nonprotein amino acids, some steroids, some sesquiterpenes, the carbon glycosides of flavones, and even some fatty acids (such as petroselenic acid of the Umbelliferae, Araliaceae, and Simarubaceae) are substances whose taxonomic distributions may reflect one to several independent evolutionary appearances.

As a general guide to the systematic evaluation of comparative chemical data, one may use the "percentage of frequency rule" (*3*). In principle, this rule states that the taxonomic distribution of a member of a group of biosynthetically related substances is inversely related to the chemical complexity of the member. Therefore, the more complex members of a chemical series occur in taxa which are evolutionarily more advanced. As long as the principle is not applied as a rigorous criterion for phylogenetic positioning it is useful. However, numerous examples, involving loss mutations and other evolutionary mechanisms, may be called to mind which limit the reliability of the percentage of frequency rule. Figure 2 illustrates the broad application of the rule to a few selected groups of phenolic compounds, all of which are considered to have a close biosynthetic relationship. An apparent contradiction to the percentage of frequency rule is encountered among quinones. Excluding coenzymes (for example, ubiquinone), the monocyclic benzoquinones have a more



Nootkatin (conifers)

Stipitatic acid (*Penicillium*)

Fig. 1. Chemically similar tropolones, which occur in conifers and fungi.

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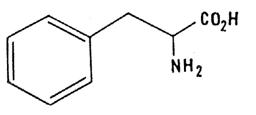
limited distribution than the bicyclic naphthaquinones, while the tricyclic anthraquinones are the most widespread taxonomically. However, there is yet no evidence that these three groups of quinones are biosynthetically related, and if they are not, the principle of the rule is not violated in this example.

Numerous examples may be cited to illustrate the fact that knowledge of biosynthetic routes and genetic mechanisms and even general physiology can be used to reinforce the purely distributional data of comparative chemistry. Such knowledge will contribute immensely to the validity of the systematic interpretations based on such distributions.

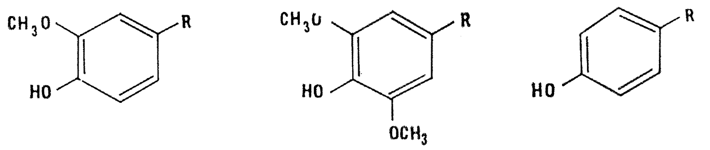
Biosynthetic Pathways

There are two different pathways for the biosynthesis of the amino acid, lysine: α-aminoadipic acid is the precursor in one, and diaminopimelic acid in the other (Fig. 3). As shown by Vogel (*5*), the alternate pathways to lysine are indicative of taxonomic correlations. For example, bacteria, green algae, flowering plants, and some of the phycomycetes (*Saprolegnia* and *Achlya*) utilize diaminopimelic acid to form lysine, while *Euglena gracilis* and most fungi (including other Phycomycetes) form lysine from α-aminoadipic acid. The distributions of these pathways support broad taxonomic groupings based on other lines of evidence, but they also suggest that the fungal class Phycomycetes is a polyphyletic unit. At this time, however, it is virtually impossible to equate biochemical and other types of data within higher taxonomic categories, and frequently the systematist has no recourse but to exclude the biochemical data.

The rosewood genus, *Aniba,* produces a number of secondary compounds which are derivatives of α-pyrone. Although the mode of biosynthesis of these compounds is not fully established, the patterns of substitution of the naturally occuring α-pyrones suggest that these compounds are formed by way of the shikimic acid pathway (the general pathway to aromatic compounds) with the addition of two acetate units comprising the heterocyclic ring (carbons at positions 2, 3, 4, and 5, in Fig. 4). On the basis of this putative mode of synthesis, the 4-methoxylated α-pyrones are the primitive types while α-pyrones with no oxygen function at position 4 (for ex-



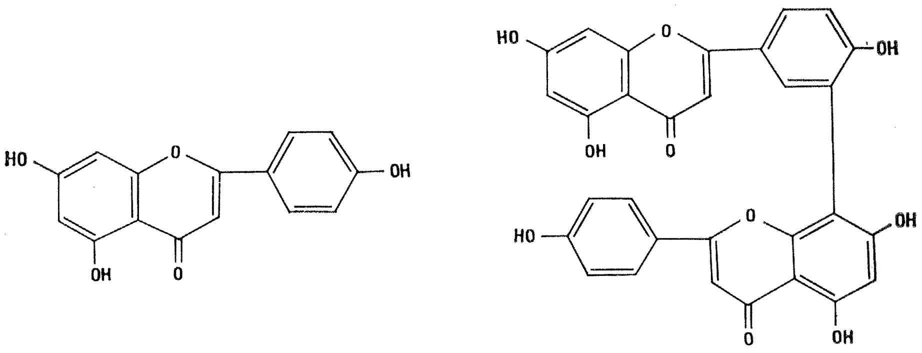
Aromatic amino acids
(all plants)



Guaiacyl-type lignins (vascular plants)

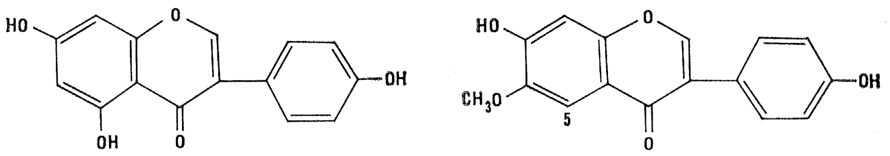
Syringyl type lignins (angiosperms and a few gymnosperms)

p-Hydroxyphenyl type lignins (grasses)



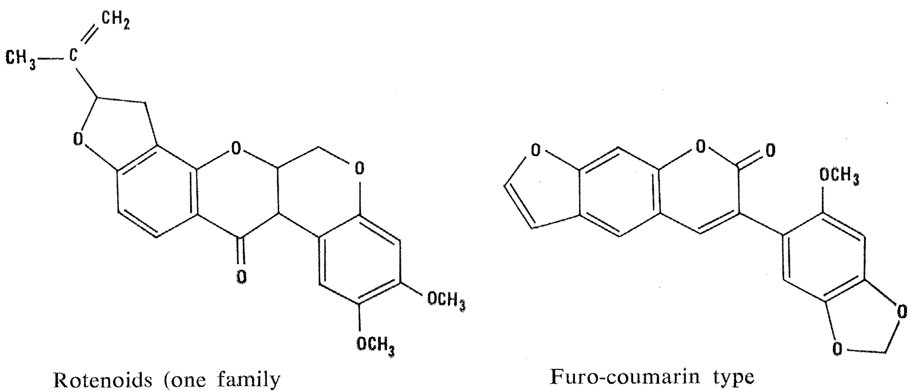
Flavonoids (ferns, gymnosperms, angiosperms)

Biflavonyl type (some gymnosperms and Casuarina)



Isoflavonoids
(four families of angiosperms)

Isoflavonoid type lacking
C_5 oxygen function
(one family, Leguminosae)*



Rotenoids (one family of angiosperms, Leguminosae)

Furo-coumarin type (one tribe, Phaseoleae, of the family, Leguminosae) †

Fig. 2. The "percentage of frequency rule" as applied to certain biosynthetically interrelated aromatic compounds. Proceeding down and to the right, structures show, generally, increasing chemical complexity and decreasing taxonomic distribution. [*J. B. Harborne, O. R. Gottlieb, M. T. Magalhaes. *J. Org. Chem.* **28**, 881 (1963); † L. Crombie and D. A. Whiting. *J. Chem. Soc.* 1569 (1963)]

ample, phenylcoumalin) are evolutionarily advanced, though structurally simpler. It is noteworthy that, according to this rationale, the distributions of α-pyrones among species of *Aniba* are in accord with the phylogenetic treatment of the genus based on the gradual reduction and suppression of verticils in the androecium (*6*).

A slightly different situation is exemplified in the benzo[*c*]phenanthridine alkaloids, such as chelidonine, which occur together with alkaloids of the berberine type in certain species of the angiosperm families Papaveraceae and Rutaceae. Structurally, these two types of alkaloids appear to be rather different and their simultaneous occurrence in two species is therefore doubly indicative of relationship. However, it was established recently (*7*) that chelidonine is probably derived from berberine by rearrangement, as illustrated in Fig. 5. In the alternative scheme, two molecules of 3, 4-dihydrophenylacetaldehyde condense to yield the benzo[*c*]phenanthridine nucleus, and the alkaloids are derived independently of berberine (*6*). Thus, the degree of taxonomic significance is dependent in part upon the presence or absence of a direct biosynthetic relationship between the compounds concerned.

In the legume genus *Lathyrus,* many species produce toxic substances causing a condition known as lathyrism. There are two forms of lathyrism, skeletal and neural, presumably brought about by different chemical agents, and the two lathyrus factors do not occur together in the same species. Recently, these factors were identified as β-aminopropionitrile (causing skeletal disorder) and L-α,γ-diamino butyric acid (causing neural disorder). Subsequently, the pathway depicted in Fig. 6 was postulated in which the two lathyrus factors were related to the common precursor, β-cyano-L-alanine (*9*). Still later, the hypothetical precursor was isolated from two species of the genus *Vicia* which is closely related to *Lathyrus* (*10*).

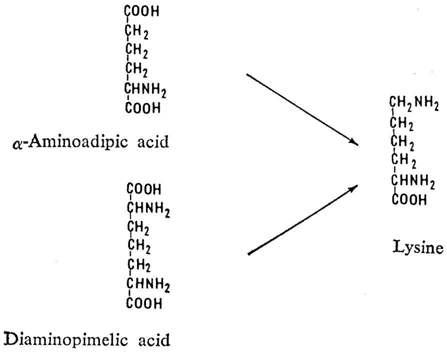


Fig. 3. Alternate biosynthetic pathways to lysine.

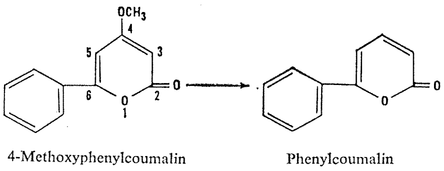


Fig. 4. The biosynthetic route to α-pyrones produces, initially, substances with an oxygen function at the carbon atom in position 4. The chemically simpler phenylcoumalins are, therefore, metabolically more advanced.

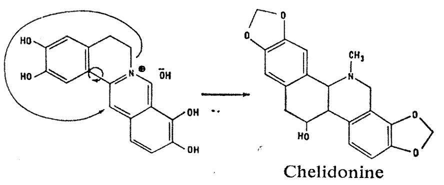


Fig. 5. The proposed (*16*) biogenetic scheme for the formation of chelidonine from a berberine skeleton.

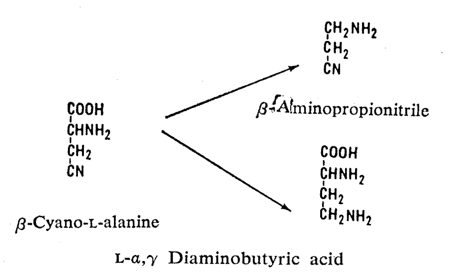


Fig. 6. Alternative hypothetical routes to lathyrus factors.

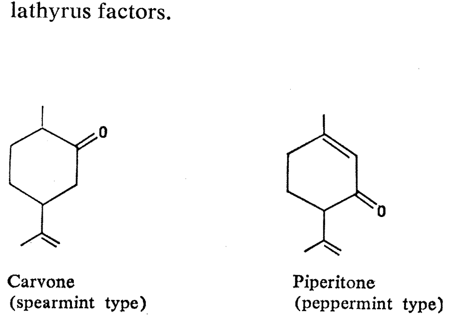


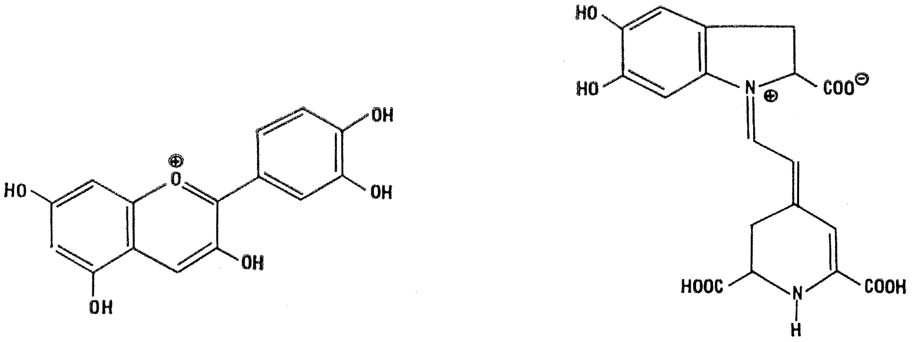
Fig. 7. Two types of terpenes which are mutually exclusive among mint species.

Genetic mechanisms

A single pair of genes governs the pattern of oxygen function substitution in mint oils. For example, the presence of the dominant allele yields the spearmint type (2-oxygenated-*p*-menthane), while in the absence of this allele the peppermint type is produced (3-oxygenated-*p*-menthane) (Fig. 7). The two types of mint oils are never found together in the same plant. Corresponding series of compounds exist in the diverse species which comprise the spearmint line and the peppermint line. Finally, another group of species, the lemon mints, produces only noncyclical terpenoid substances such as citral and linalool (*11*). This last group of compounds is considered to be representative of the ancestral mint oils from which the spearmint and peppermint derivatives formed two independent series. Among horticultural plants, in which loss mutations are frequently preserved in selected cultivars, the recessive forms are generally derived from the dominant. Among mint species, however, the genetic dichotomy seems to have been initiated long ago, and this type of generalization cannot be assumed to apply necessarily in this instance.

Physiological Considerations

Although thousands of secondary compounds are produced by plants, only a few of them have been shown to have any specific primary physiological role. However, since natural selection takes part in the evolutionary emergence of a new type of molecule, the survivors of such natural selection must have some functional significance, even though it be slight. A new reaction may be truly novel, conferring some unique advantage, or it may merely provide a similar but more efficient mechanism than formerly existed. In the latter instance, the less efficient mechanism may be replaced and, perhaps, genetically lost. Since mutants lacking a given secondary compound generally survive and often flourish, it is evident that the adaptive value of the compound lost was in that instance less than 100 percent. Although the present adaptive value of a particular substance may be slight or even negligible, selective forces in the past may have been quite different. Anthocyanin pigments, responsible for the color of flowers, constitute an excellent example of a group of compounds whose role in the plant is inadequately explained by merely assuming that the pigments attract insects. In many species these pigments are found in leaves, in stems or even in roots; the pigments also occur in ferns and grasses, for which insects are not important agents of spore or pollen dissemination. Thus, other physiological



Cyanidin cation

Betanidin

Fig. 8. Aglycons of a typical anthocyanin and betacyanin; two chemically distinct flower pigments.

roles have at times been advanced for these compounds. The fact of greatest interest in the present discussion is that anthocyanins have been replaced, in one large group of plants comprising several related families, by betacyanins (*12*), substances which are chemically quite different. The two types of pigments are compared in Fig. 8.

These two types of pigments have never been found together, even within the same plant family, although flavonoids which are related to the anthocyanins may occur together with betacyanins in the same plant. Until recently, betacyanins were referred to as "nitrogenous anthocyanins," and they were assumed to be flavonoid in character. It is noteworthy, though probably coincidental, that both types of pigments appear to be derived metabolically from pathways to the aromatic amino acids. It is also of special interest that the most prevalent betacyanin glucoside, betanin, and the commonest anthocyanin, cyanidin glucoside, have maximum absorption spectra within 5 $m\mu$ of each other. The conditions required for the synthesis of betacyanins have not been studied intensively, but preliminary data indicate that they differ greatly from the conditions required for the synthesis of anthocyanins. For example, the formation of anthocyanins is essentially dependent on light and is aerobic, while in beet seedlings, betacyanins form in the absence of both light and oxygen.

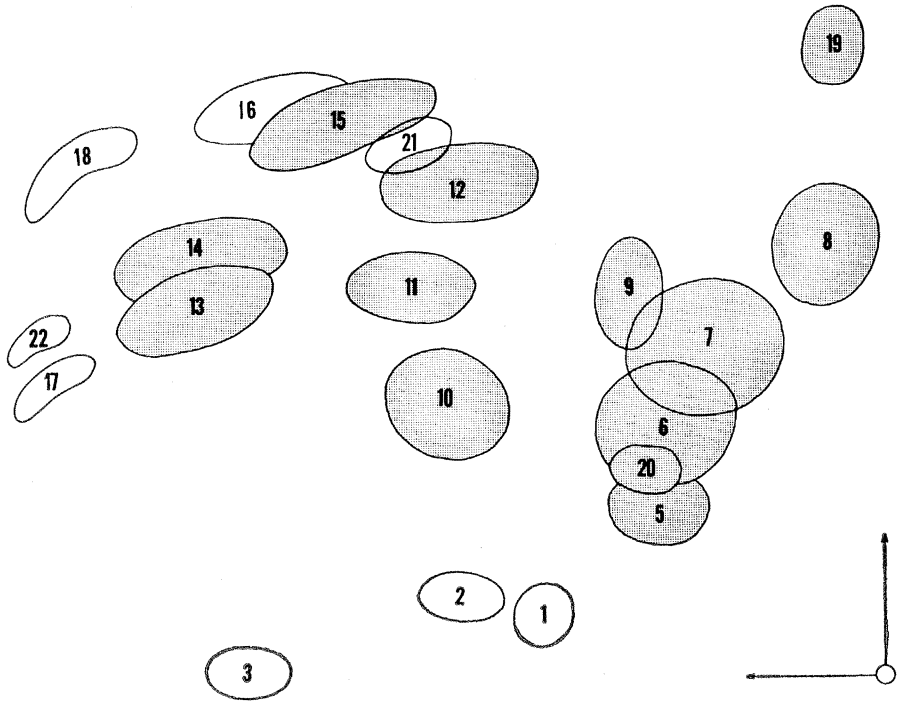


Fig. 9. Typical chromatographic pattern of whole plant extract of *Spirodela oligorhiza* in axenic culture (Hutner's solution, one-third strength, at 24°C and 16,500 lu/m^2). Shaded spots represent substances tentatively identified as flavonoids on the basis of spectral data.

Thus, while the chemical properties and the conditions required for synthesis of these two classes of pigments differ, they are mutually exclusive in a single plant, and their absorption spectra match closely. Therefore, it is tempting to suggest that the compounds have the same role in the plant, a role which is dependent upon their properties as pigments. Moreover, the presence of related flavonoids together with the betacyanins, and the restricted distribution of the latter, suggest that the roles of the anthocyanins and of other flavonoids are different, and that it was the betacyanins which were selected for in the process of evolution. The specific taxonomic distribution of betacyanins bears out this theory satisfactorily (*13*). Betacyanins are limited in their distributions to ten families usually grouped in the Centrospermae: Amaranthaceae, Basellaceae, Cactaceae, Chenopodiaceae, Didieraceae, Aizoaceae, Nyctaginaceae, Phytolaccaceae, Portulacaceae, and Stegnospermaceae. The family Caryophyllaceae, placed by most workers in the Centrospermae, is exceptional in that betacyanins have never been shown to be present. Anthocyanins are common in the family, however. It is important to recognize that of the families listed, not all have been relegated to the Centrospermae by all taxonomic workers. Some recent additions to the group containing betacyanin are interesting in this respect. For example, the Didieraceae, a family of spiny xerophytic trees restricted to Madagascar, are now known to contain betacyanins (*14*). The taxonomic affinities of this family have been considered generally as being with the Sapindales, but at least one worker (*15*) has placed the Didieraceae in the Centrospermae. The finding of betacyanin in *Stegnosperma*, which has been considered as comprising the sole genus of the subfamily Stegnospermatoidiae of the Phytolaccaceae (*16*), is notable. Hutchinson (*17*) recently elevated the subfamily to familial status and related it to the woody order, Pittosporales, of the Lignosae, which he believes to have only remote relationships with the herbaceous Centrospermae. Betacyanins thus appear to be taxonomically significant at the higher categorical levels. It is unusual to draw conclusions of such major systematic importance from the taxonomic distribution of a single chemical character. Yet, the facts surrounding the betacyanin pigments suggest the probability that these compounds evolved only once, that they succeeded in replacing or substituting for the anthocyanins within certain evolutionary lines, and that those taxa in which the betacyanins occur are phylogenetically related.

Biochemical Systematics in Baptisia

Studies of chemical compounds occurring in the genus *Baptisia* (family Leguminosae) have exposed new applications of comparative chemical data: to the study of natural hybridization, including the validation of hybrids, population structure, gene flow, and introgression. This work began in 1959 when Turner and Alston demonstrated, with the aid of paper chromatography, the recombination of six species-specific compounds in putative hybrids between *Baptisia leucophaea* and *B. sphaerocarpa* (*18*). Subsequently, this type of work was extended to include other *Baptisia* hybrids with more complex associations, and the chromatographic techniques were improved so that more compounds could be detected.

The genus *Baptisia* consists of approximately 20 species of herbaceous perennials restricted in their distributions to the central and eastern United States. Five species are limited essentially to an area between the Mississippi River and central Texas, Oklahoma, and Kansas, and the remainder of the species are concentrated in the southeast with at least one species, occurring as far north as upper New England. The various species of *Baptisia* are morphologically distinct, often exceptionally so, but natural hybridization is frequent among species occurring together. Many of the hybrid combinations involve strikingly different species; hence it is likely that synthetic hybrids can be produced between most, if not all, of them.

Species of *Baptisia* contain a large variety of secondary compounds which can be separated effectively by paper chromatography, so that individual plants can be analyzed; for example, flavonols, flavones, flavonones, isoflavones, and anthocyanins have been tentatively identified among the flavonoid components from one species, *B. sphaerocarpa*. Different species of *Baptisia* yield characteristic two-dimensional chromatographic patterns from which accurate taxonomic identifications can be made. There appear to be three basic chromatographic patterns, each with a number of variations. We do not yet know whether these different patterns reflect subgeneric phylogenetic associations or coincidental resemblances, although the similar patterns of certain white-flowered species are matched by a corresponding distinctive morphological unity among these species.

Early in our investigations the question of individual variation in the chemistry of the plant, whether ecotypic, genetic, or developmental, was raised repeatedly. Recent work on the minute aquatic plant, *Spirodela oligorhiza* (Lemnaceae), has disclosed only relatively minor differences in the chromatographic patterns of the same strain grown under sterile conditions upon various defined media (Fig. 9; Table 1) (*19*). Furthermore, strains of this species acquired from various sources yielded nearly identical chromatographic patterns. The flavonoid components of *Spirodela oligorhiza* are numerous, they are present in large amounts, and they differ markedly from those of *Spirodela polyrhiza*, a presumably closely related species.

While it is impossible to predict the limits of variation in one species by extrapolation from another set of data, it is likely that Brehm (*20*), through his analysis of *Baptisia leucophaea* var. *laevicaulis*, has established a reliable general guide to the extent of variation which may be anticipated in *Baptisia*. The chromatographic patterns, representing mostly phenolic (including flavonoid) substances, are extremely reliable taxonomic guides, and many of these compounds are constantly present in relatively similar quantity. Other substances, usually minor components, are less apt to be constant. The chromatographic patterns of leaf and flower

Table 1. Variations observed in the chromatographic patterns of strains of *Spirodela oligorhiza*, obtained from different sources and grown asceptically under a variety of conditions. Spot numbers are correlated with those of Fig. 9. Symbols: x, concentration of substance equal to that of control; >, concentration greater than that of control; <, concentration less than that of control; —, substance not detected.

Conditions of culture or state of growth	Spot number															
	1	2	3	5	6	7	8	9	10	11	12	15	16	17	18	19
Yale University																
Control*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Methionine (1×10^{-3} M)	x	<	x	<	<	x	x	x	x	x	<	x	x	x	x	—
Indole acetic acid (50 mg/l)	x	x	<	x	x	x	x	x	x	x	x	>	x	x	x	<
Benzimidazole (400 mg/l)	x	x	<	x	x	<	—	x	x	x	x	x	x	x	x	—
Gibberellic acid (1 mg/l)	x	x	x	x	>	x	x	x	>	>	x	>	x	x	x	<
Thiourea (3×10^{-2} M)	>	x	>	x	>	x	x	x	>	>	>	>	x	x	x	<
Kinetin (1 mg/l)	x	x	x	x	x	<	x	x	x	x	x	>	x	x	x	x
⅓ Hutner's + milk (1%)	x	x	x	x	x	<	x	x	x	x	<	<	x	<	x	<
30 C; 3300 lu/m^2	x	<	x	<	>	x	x	x	x	>	>	>	x	x	x	<
Sucrose, 21°C; 1100 lu/m^2	x	<	x	<	x	<	x	x	x	x	x	x	x	x	x	—
Sucrose, pH 4.0	x	<	x	<	x	<	x	x	x	>	x	x	x	x	x	<
Sucrose, pH 8.8	x	<	x	<	>	<	x	x	x	x	x		x	x	x	<
⅓ Hutner's	x	<	<	<	x	<	x	x	x	—	x	x	x	—	x	x
Berkeley, California																
Peak growth	x	x	x	<	x	<	x	x	x	x	<	<	x	x	x	>
Aged	x		x	x	>	x	x	x	x	x	x	x	x	<	x	x
Austin, Texas																
Peak growth	<	x	x	<	>	x	x	<	<	x	<	x	x	x	x	x
Kill Devil Hill, North Carolina																
Peak growth	x	<	x	<	x	<	>	x	x	x	x	x	x	x	x	x
Sao Paulo, Brazil																
Peak growth	x	<	x	<	x	x	x	x	x	x	x	x	x	x	x	x

*Control: Hutner's solution, 1/3 strength, at 24°C and 16,500 lu/m^2.

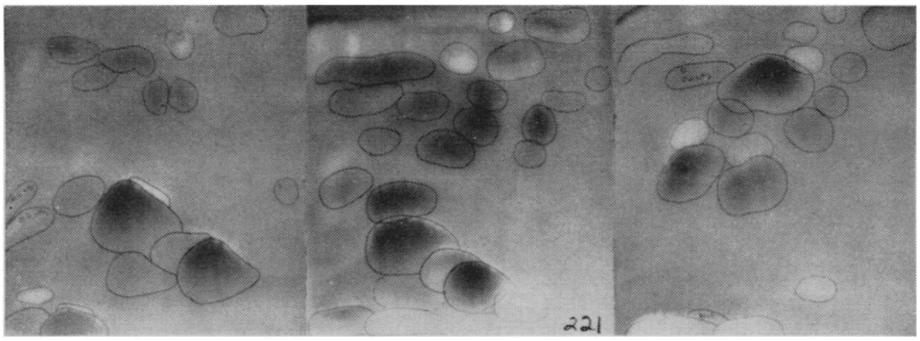


Fig. 10. Two-dimensional chromatograms of leaf extracts of two species of *Baptisia; B. nuttalliana* (left), *B. leucantha* (right), and their hybrid (center). The hybrid's chromatographic pattern represents essentially a composite of the patterns of the two parental species.

extracts are generally similar, but the patterns may be distinguished in every instance. Chemical races definitely exist in this taxon. In contrast to the phenolic substances, alkaloids of *B. leucophaea* var. *laevicaulis* vary unpredictably among individual plants, and these compounds exhibit major quantitative and qualitative changes during the life of the plant.

Natural Hybridization

Natural hybridization has been the most thoroughly investigated facet of the *Baptisia* program to date. Reasons for this are that the techniques which were available in the early stages were immediately applicable to this type of study, and the study itself continually uncovered more complex and, indeed, more exciting possibilities. Simple documentation of putative hybrids by chromatography is not difficult in most instances when only two species are encountered together (Fig. 10). So far, 11 different hybrid combinations have been validated by the correlative use of morphological and chemical data. Other hybrid combinations have been reported by other investigators (*21*). The most complex population described so far, in a field near Dayton, Texas, (*22*), contained four pure species and all six possible hybrid combinations plus bizarre individuals of indeterminate origin. In the Dayton population, documentation of the six types of hybrids by morphological criteria alone was seemingly impossible, but the combined morphological and chromatographic data furnished adequate proof of the existence of the six types of hybrids. In Georgia, two white-flowered species, *B. alba* and *B. pendula*, are found together with the yellow-flowered *B. lanceolata* and certain hybrids. It is virtually impossible to ascertain on the basis of morphological characters which white-flowered species is involved in a particular hybrid. However, the chromatographic patterns of these particular white-flowered species differ greatly, and the origin of a given hybrid is easily established from these patterns. Both hybrids have now been validated (*23*). Elsewhere in Georgia, the species *B. lanceolata* and *B. perfoliata* occur in a large sympatric population which yielded seven hybrids. Chromatographic data indicated that one of the seven hybrids was derived from *B. perfoliata* and *B. alba* although the latter species was not observed at the site. The reliability of the chromatographic patterns is sufficient to allow the prediction that *B. alba* was present somewhere near that population and the next year *B. alba* was collected within 5 miles of the site. These examples could be supplemented by others and serve to establish the value of chromatographic criteria in the recognition and validation of hybrids.

Population analysis may also be reinforced through the use of chromatographic techniques. When three species occur together and are hybridizing rather complexly, a morphological hybrid index is difficult to construct. However, the species-specific compounds distinguishing both species tend to be inherited as dominants in the hybrid, which therefore exhibits a complex pattern, a summation of two characteristic species patterns. Although we do not know precisely what the chemical makeup of a backcross type would be, it is likely that all compounds of one species would be present along with some components of the other species—the number and particular array varying with each individual. Of course, if each species-specific compound were the product of a complex series of reactions, each step of which was governed by a species-specific enzyme, one or another link in the sequence might be broken in every backcross and few components of the second species would appear.

One trihybrid population involving *B. leucophaea, B. sphaerocarpa,* and *B. leucantha* was investigated recently, and by use of a three-way morphological hybrid index it was deduced that the population was extremely heterogeneous, with the three genomes frequently incorporated to varying extents into a single plant. However, a chromatographic analysis of the same plants disclosed mostly hybrids between *B. leucophaea* and *B. sphaerocarpa*, and between *B. sphaerocarpa* and *B. leucantha*, plus a very few types suspected as being backcrosses. No hybrids between *B. leucophaea* and *B. leucantha* were encountered although they could have easily been recognized chromatographically, and no evidence of complex hybridization was forthcoming. A morphological reappraisal of the total population structure supported the interpretation based on the chromatographic data, since most plants in the field were apparently pure species (*24*).

The species *B. leucantha* and *B. sphaerocarpa,* which hybridize readily, have quite different chromatographic patterns with few common constituents. Therefore, this pair of species was selected to support a chromatographic study of hybrids (*25*). The objectives of the study were to compare morphological and chromatographic patterns in F_1 hybrids, to attempt to detect backcross types chromatographically and to establish the nature and extent of backcrossing in natural populations in which only these two species occur. The results of this investigation clearly demonstrated that when hybridization is occurring between only two species, the general implications of morphological and chromatographic data agree. For example, out of more than 150 individual hybrid-type plants, most appeared to be F_1's; relatively few backcross-types were found and of these, most were apparently F_1's crossed to *B. sphaerocarpa*. Despite the general agreement, there were occasional sharp differences in the chromatographic and morphological interpretations of single plants. If the group of F_1 hybrids was accurately determined, there was considerable chromatographic variation among the group. The chromatographic variation of the putative F_1's exceeded that of the pure populations of the parental species. The group of putative backcross-types was arbitrarily selected, with no assurance that the break between F_1 types and backcross types was accurately determined. These putative backcross types were variable but contained essentially a full complement of the components of one or the other species. We are now extending this type of population analysis. For example, one large population of *B. leucophaea* and *B. sphaerocarpa,* together with numerous hybrids, has been staked out in a plot 90 meters square (90,000 ft^2) and all plants within the plot are being collected (over 2000) as they flower. The onset of flowering in the two species is different though their flowering periods overlap. The number of shoots emerging from the rhizome of a plant at ground level provides a rough approximation of the age of these long-lived perennial plants. The exact location within the plot of each plant is being noted, as well as the time of flowering and the number of shoots emerging, so that a comprehensive morphological and chromatographic analysis can be made. The seedlings, the origins of which cannot be adequately interpreted on the basis of morphology, have the characteristic leaf chemistry, and may therefore be analyzed chromatographically. All of these data, when collected and correlated thoroughly by means of digital computers, should yield facts about the current pattern of hybridization, in fact, of gene flow, in the population. We shall even be able to plot the density gradients for particular characters which distinguish the species, and make crude chronological extrapolations utilizing the data from seedlings and from older plants of various ages.

Chemical evidence of introgression should be more definitive than morphological evidence in *Baptisia.* Chromatographic screening in areas adjacent to where hybridization is occurring may disclose introgression, but this type of study has not yet been made. When a large number of hybrid derivatives are available, correlations may be revealed between individual compounds to establish possible cohesion of chemical characters in the sense of Clausen (*26*). Analysis of synthetic backcross-types could be applied to determine whether such cohesion reflected natural selection or linkage or a resultant of these two forces. The species *B. leucophaea* and *B. sphaerocarpa* are best suited for such work because of the occurrence of large numbers of hybrids and the presence of several distinctive chemical components in each species.

Genetic Interactions

Over 100 compounds of phenolic nature have already been disclosed among the species of *Baptisia* so far examined and, without doubt, many others will be found. The plant constituent most thoroughly studied, with respect to biochemical genetics, has been one of the groups of phenolic compounds, the anthocyanin type of flavonoid (*27*). Despite the background of classical work in the field, there is little known concerning genetic interactions among various subtypes of flavonoids. In *Baptisia,* species differing in a variety of flavonoid subtypes and other phenolic components may be successfully hybridized, so that numerous metabolic interactions can be analyzed genetically. Hybrids can be constructed theoretically from a choice of many parental chemical patterns, and more complex hybrid derivatives can be produced ultimately. Most studies of the biochemical genetics of flower pigments have been carried out on ornamental plants, and the data are not directly relevant to the evolution of the plants concerned. The genetic studies in *Baptisia,* in contrast, are relevant to questions of sequential or parallel evolution of genetic regulatory mechanisms which govern, independently in numerous species, the same general class of compounds. Not only do the specific chemical patterns provide clues to patterns of evolution within the group of species, but so does their mode of inheritance.

The general question of genetic regulation of the chemistry of an organism has been raised in an unusual way by virtue of certain peculiarities manifest in *Baptisia* hybrids. For example, the flowers of *B. sphaerocarpa* contain four distinctive components in large quantities. These compounds are probably flavonol glycosides, but they have not been completely characterized. In all hybrids between this species and *B. leucantha* which have been examined (over 100 plants) these four compounds are prominent in the leaves as well as in the flowers, although *B. leucantha* lacks the compounds, and *B. sphaerocarpa* has the compounds only in the flowers. Other hybrids, for example, between *B. sphaerocarpa* and *B. leucophaea,* do not contain these compounds in the leaves (*28*). It was suggested that *B. sphaerocarpa* has developed a regulatory mechanism which limits the distribution of the compounds to flowers, but in certain hybrids the mechanism is upset and the leaves also accumulate the compounds. In a genetically closer cross (that is, one in which *B. leucophaea* is one parent) the regulatory mechanism of *B. sphaerocarpa* is still effective. In addition to the four compounds noted here, a major leaf component of *B. leucantha* occurred occasionally as an additional compound in the flowers of hybrids of *B. leucantha* crossed with *B. sphaerocarpa* (*25*).

A further interesting feature of the four "hybrid type" compounds discussed herein is their reappearance in leaves and flowers of two different hybrids of the southeastern species, *B. alba* and *B. perfoliata,* although neither parental species contained the compounds in either organ. *Baptisia alba* is a white-flowered species which appears, on the basis of its chromatography, to be an advanced member of the *B. leucantha* group, while *B. perfoliata* is chromatographically similar to *B. sphaerocarpa. Baptisia perfoliata* is a bizarre and probably advanced species; it also has some morphological features (for example, fruit characters, and the color and texture of the leaves) resembling those of *B. sphaerocarpa.* In this hybrid, the compounds are truly hybrid-specific, but the mechanism may, indeed, be homologous with that which governs the distribution of the hybrid-type substances found in the cross between *B. sphaerocarpa* and *B. leucantha.*

Comparative Chemical Studies

It is our intent to study extensively the comparative chemistry of *Baptisia* and related genera, in connection with the systematics of these plants. The specific associations of flavonoids, and of other classes of chemicals which appear among the various species, may provide clues to species relationships. Some flavonoids which we have encountered, such as luteolin and cyanidin glycosides, are extremely common and are not in themselves of major taxonomic interest. However, as pointed out

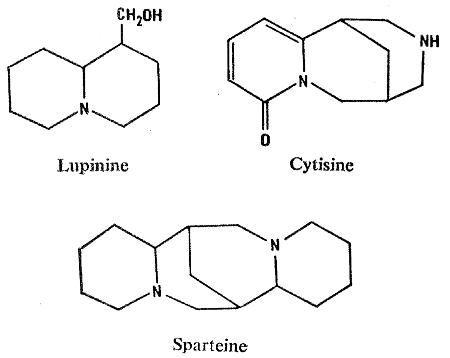


Fig. 11. Representative lupine alkaloids from *Baptisia* species.

by Harborne (*29*), even when the aglycone is a common flavonoid the glycosides are still likely to be of systematic importance. It has already been established that one major distinction between *B. leucophaea* and *B. sphaerocarpa* is based on the presence of different glycosidic derivatives of luteolin in the two species. However, these same two species have differences among their anthocyanin aglycones (anthocyanidins). One group of flavonoids found in *Baptisia*, the isoflavones, is exceedingly rare, having been reported sporadically in only four plant families: the Leguminosae, Iridaceae, Moraceae, and Rosaceae. Within the Leguminosae, isoflavones occur only among certain tribes of the subfamily Papilionoideae, including the genus *Baptisia* and the closely related genus, *Thermopsis*. In the Leguminosae the distribution of these isoflavones is significant above the generic level, but the specific patterns of substitutions of these compounds within the genus *Baptisia* should also be of systematic interest. Another group of substances, the rotenones, are now believed to be biosynthetically related to the isoflavones. Among the groups which produce isoflavones, only the Leguminosae contain rotenones. We have not yet succeeded in isolating rotenones from *Baptisia*, but since it is reported that *Baptisia* shoots are used as insect repellents, it is probable that the plants do contain rotenones. Among other classes of flavonoids, the presence in *Baptisia* of the somewhat uncommon flavanones has now been confirmed; the common flavonols are also represented. Therefore, flavonoid chemistry in *Baptisia* promises to be sufficiently complex to yield interesting comparative chemical data.

The lupine alkaloids represent another completely unrelated group of compounds which are numerous and widely distributed in *Baptisia* (Fig. 11). These compounds are found in the Leguminosae and a few scattered families taxonomically distant from this family. In Leguminosae, the alkaloids occur only in the tribes Sophoreae, Genisteae, and Podalyrieae, of the subfamily Papilionoideae. The alkaloids have been studied intensively in the genus *Lupinus* by Polish workers, who have been interested in their chemistry, biosynthesis, physiology, genetics, and systematic importance (*30*). A few species of *Baptisia* have been investigated and all are rich in alkaloids of this type; for example, nine different alkaloids occur in *B. leucophaea*. Since these alkaloids appear to be quite labile in the plant, and chromatographic studies have shown the individual plants of a population to vary greatly in the relative and absolute amounts of alkaloids present, the compounds have been of no value in our studies of natural hybridization.

Amino acids have not proven to be of major general systematic value despite early interest in them as taxonomic guides. However, the family Leguminosae has proven to be exceptional in possessing a large number of non-protein, often unusual, amino acids. In some instances these amino acids are limited to a few species of a single genus, for example, tingitanin has been found only in *Lathyrus* (*31*). These non-protein amino acids have, on occasion, been of definite systematic importance (*32*). A number of non-protein amino acids have been detected in *Baptisia*, but no effort has yet been made to characterize these compounds.

From this discussion, it is evident that intensive comparative chemical studies can be carried out, almost independently, on several unrelated groups of secondary compounds in *Baptisia*. Certain of these chemical groups are taxonomically restricted, but they may not be especially suited to adducing relationships within a taxonomic group. It is hoped that by studying the distributions of specific types of several differing groups of components, such as the lupine alkaloids, the effects of complex chemical parallelisms and convergences in evolution may be discovered. These phenomena frequently diminish the validity of phylogenetic speculations based on the comparative chemistry of a single group of compounds (*33; 34*).

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