

Otto Richard Gottlieb

Micromolecular Evolution, Systematics and Ecology

An Essay into a Novel Botanical Discipline

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With 80 Figures

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Foreword

For several decades botanists have been impressed by the discovery that the distribution of secondary plant substances follows the general lines of plant relationships. However, it soon became clear that little was to be gained from the study of individual compounds and their natural distribution. Therefore, more comprehensive studies were attempted in which the secondary chemistry of a major plant group was carefully studied and evaluated in the broader context of comparative phytochemistry. Holger Erdtman's admirable work on Coniferae is the foremost example of this kind. Since then, there has been an upswing in the study of the biosynthesis of secondary plant substances and it has become quite customary to make use of biosynthetic knowledge in interpreting chemosystematic evidence. Moreover, since taxonomists have insisted that use be made of all potentially available evidence for building classifications, it has been claimed that chemosystematics too should consider the whole array of constituents present in a major taxon.

However, in practice it has proved difficult to utilize fully the potential of natural product chemistry and biosynthetic studies for plant systematics and evolution, because botanists found themselves rather disorientated by the scattered, often hardly accessible chemical literature and the fact that the chemical evidence was difficult for them to evaluate!

Although the pioneering work of E.C. Bate-Smith paved the way towards a rational use of chemical data in systematics and evolution, for a long time a simple comparison between existing classifications and the distribution of chemical data prevailed, something that today might be called the "narrative approach" to chemosystematics.

It is here that the highly ambitious, dynamic approach of Professor Gottlieb comes in: he shows how much can be gained by carefully collating and organizing the available in-

formation about the distribution of secondary compounds. It is indeed embarrassing for plant taxonomists that an outsider has achieved what systematists themselves have always claimed as forming the basis for systematics and taxonomy: to bring together in a systematic fashion the available data from different fields of study. However, Professor Gottlieb's endeavour did not stop at this stage but led to a further development of methodology: a consistent biogenetic classification of chemical compounds and their codification brought the items of information together in an intelligible context and at the same time served to overcome what was for many biologists the cryptic nature of chemical formulae. The application of additional parameters led then to the formulation of very meaningful expressions of chemical advancement which, as Professor Gottlieb believes, constitute a systematic criterion that is independent of conventional, mostly morphologically based classifications.

However, the importance of the findings of this book goes well beyond the narrow frame of the classification of particular plant taxa: it is indeed highly interesting from the standpoint of evolutionary theory to see that at the beginning of each major evolutionary line there are primary precursors from which the chemical diversity within each line does originate while the precursors themselves originate via increasing blocking of reaction steps.

Perhaps the most interesting general result of the analysis is the notion that structural variation of secondary compounds conforms to a systematic and geographical continuum, which finds its expression in the structural variation of metabolites of a given biogenetic group or in the homology of biogenetic pathways. The existence of the geographical continuum is indirect proof of the adaptive, i.e. allelochemic nature of secondary compounds that have changed – normally in small steps – in their need to adapt to new environments during the spread of the respective lineages. While chemical ecology studies the mechanisms that show how chemical changes come into being, Professor Gottlieb's analysis traces the routes this evolutionary process has taken.

Professor Gottlieb's text requires careful study but those who are willing to follow the author through these pages will, I am sure, be highly recompensed by the ideas presented in this book.

Hamburg, November 1981

Klaus Kubitzki

Preface

Summary, appraisal and integration of data on chemical markers of low molecular weight is required in order to bring them into proper perspective for the classification of plants. Integration of evidence, necessary or desirable as it may be, is a complex and hazardous task, even if limited to chemical data. The number of species for which adequate chemical analyses have been published is ridiculously small, only a few percent. What this percentage amounts to exactly cannot be stated, since the definition of an adequate chemical analysis for systematic purposes is unknown. Thus in order to summon courage and force an entry into micromolecular systematics at all I had first to convince myself that as a human being I have no time to wait for the completion of a reasonably representative chemical inventory and, having acquired a basic scientific faith, must work with the available data, however fragmentary.

Given, furthermore, the present rate of alteration of the biosphere (dramatically noticeable chiefly in developing countries such as my homeland, Brazil) and the widespread regrettable notion that the simple registry of the chemical composition of a species is a routine task which will do little to further science, will there ever come the day for chemical plant systematics when one will be able to say "the time is ripe"?

Finally I can see no reason for considering micromolecules as mere auxiliary markers in substantially morphological classifications (alas they perform rather poorly in this their traditional role), while, at the same time, morphologists continue their centuries old struggle towards a natural classification; indeed each major systematist has his own system which even he himself is forced to revise from time to time. Clearly a natural classification can only come into being if morphology and allelochemics, both subject to the same

genetic and environmental constraints, are considered; if equal status is accorded to morphological and micromolecular systematics in an integrated system. The vastness and the interdisciplinary nature of such a system, which preclude its production at a stroke, form the third attenuating circumstance for this book which merely aims to sound the bell for departure in this direction.

Happily I had the company on this perilous journey of several enthusiastic collaborators. Their pertinent theses are listed in References under Rezende (after marriage Gomes 1972, 1975), Marx (after marriage Young 1975, 1979), Cagnin (1976), Temperini (1977), Rocha (1977), Silva (1978), Salatino (1979), Kaplan (1980), Bolzani (1982), Guajardo (1982), Alziati (1982), Kitagawa (1982) and Barreiros (1982). Some of them became so fascinated by the insight micromolecular systematics allows a chemist to gain into the evolution of life on our planet that their interest continued after graduation. I also wish to acknowledge the encouraging comments of Professor Dahlgren from Copenhagen University. Dr. Jensen and Dr. Nielsen, The Technical University of Denmark, provided a file of reports on the occurrence of iridoids and recommended the work reported in Chap. 7 to be based on their classification of iridoid skeletons. My interest in the *Derris-Lonchocarpus* problem was aroused by Professor Marini-Bettòlo and Dr. Delle Monache, Università Cattolica del Sacro Cuore, Rome. Chap. 14 is based on a literature survey they kindly provided and on the botanical expertise of Dr. Polhill, The Royal Botanic Garden, Kew. Dr. Gottsberger, Universidade Federal do Maranhão, São Luiz) investigates pollination ecology and was an ideal collaborator in the work reported in Chap. 16. Finally, I take pleasure in singling out Professor Kubitzki from Hamburg University whose contribution goes well beyond the special topics to which his name is appended, since he initially inspired me to write this book and then proceeded to read, comment and correct the entire manuscript.

São Paulo, June 1982

Otto R. Gottlieb

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Allelochemics as Systematic Markers

Life, as we know it, is inconceivable without hydrogen, carbon, nitrogen, oxygen, phosphorus, sulphur etc. The production of the heavier of these elements requires several billion years of "cooking" time in the interior of a star. The time lapse from the "Big Bang" to the final gravitational collapse of a universe, however, is, according to Einstein's General Theory of Relativity, completely dependent on mass. Life is thus related, through time, with mass and cannot exist in a universe significantly heavier or lighter than our Universe's 10^{22} stars (Eccles 1979). The fact that the existence of a star, which may be up to 8 billion light years from us, has a bearing on life here, makes any statement to the effect that the existence of life depends on the environment on planet Earth sound like a truism.

Indeed, the continued existence of each organism is dependent on the superstructure of the ecosystem in which it occurs. Understanding the functioning of such ecosystems, a problem that is of crucial importance, has been helped only to a limited extent by biological, let alone taxonomic work. Clearly, if we are to advance more rapidly in this vital field, new methodology seems to be needed (Raven et al. 1971). I interpret this statement to include new systematic criteria, and wish to justify initially my choice of micromolecules.

Why Micromolecular Systematics?

Ideally a natural classification should consider the different levels of manifestation of the genotype (Fig. 1.1) as criteria for progressively lower hierarchical levels in an integrated system. Such a target is not yet within reach and at present we still have to choose one particular level as a classificatory criterion. If understanding of functioning of living organisms is a motivating factor of one's endeavour, allelochemics, chemical signals produced by plants and animals for interaction with organisms other than the producer (Janzen 1978), should not be ignored for classificatory purposes.

Genotype

Phenotype

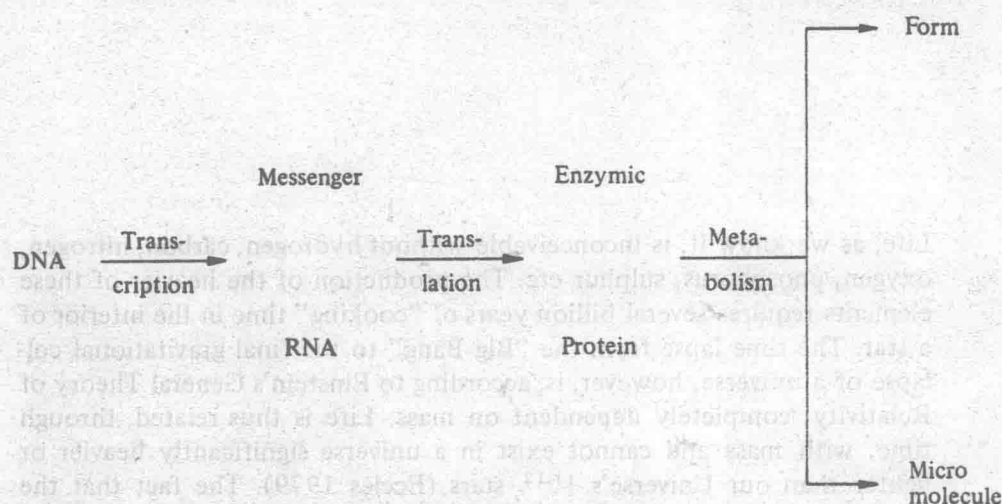


Fig. 1.1. Levels of manifestation of the genotype

Whether these compounds are really relevant for the study of plant classification and evolution is of course open to enquiry, and is the main theme of the following chapters.

Signals must be transmitted through space and the compounds involved in the mediation of the interactions between organisms are consequently usually small, somewhat soluble or volatile compounds: micromolecules, often also called secondary metabolites. Their role as allelochemicals has hitherto been proven only in a few instances. However, evidence is accumulating to support a primarily ecological role for micromolecules both as mediators of interactions between plants and their associated biota and as protective agents against physical environmental stress (Harborne 1977 b, Rhoades 1979). Indeed it would be difficult to understand the enormous structural variability of secondary metabolites solely on account of their metabolic significance or their function as storage compounds or hormones. Nevertheless it is still a matter of debate whether or not all of these compounds must possess, today or in the past, an adaptive significance.

It will not go unnoticed by the reader that it is precisely this dependence on environmental conditions that has, in the past, discouraged the use of allelochemicals as systematic markers. Indeed it continues to be stated, e.g. by Harborne (1980), that characters subject to strong selective pressure are unsuitable for taxonomic purposes. For this reason, most of the fundamental research in chemosystematics to date is concerned with

macromolecular compounds: nucleic acids (DNA) and homologous proteins (cytochrome c, plastocyanin, ferredoxin, haemoglobin) (Smith 1977). These levels are closely associated with the manifestation of the genotype, but environmental factors only indirectly influence the genotype (Takhtajan 1973). While this may be true, it cannot be emphasized strongly enough that any outcome of such macromolecular studies will have little bearing on the problem at hand: the understanding of ecological interactions, the sole basis for an approach to interference with nature without causing irreversible damage.

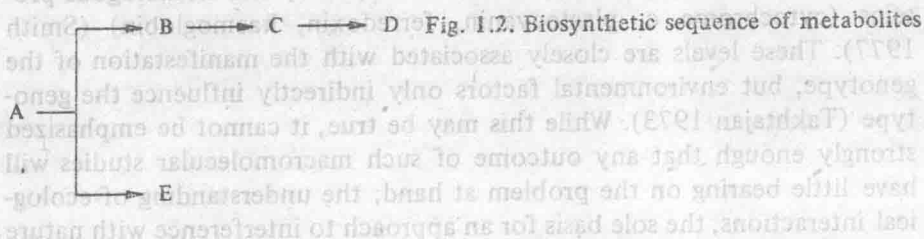
Thus, if difficulties are involved in the application of micromolecular characters to systematics one simply has to face, analyse and understand them. When starting on this task with a survey of the literature we arrived at an amazing conclusion (Rezende et al. 1975): although biochemical systematics has been a distinct field of study for over 15 years, it continues substantially to be an art, in the sense that data, mostly of the presence (+)/absence (–) type, are adduced by totally subjective methods to consolidate or complement morphological classifications. What are the reasons for the lack of fundamental knowledge which would transform our art into a scientific discipline (Gottlieb 1980a)?

The evolutionary classification of plants has a predictive value (Cronquist 1968), i.e. a better than random chance exists that certain characters of an as yet unstudied taxon will fall into the pattern which has been established for these characters by the study of a relatively limited number of its components. The closer the evolutionary relationship of studied taxa, the higher the chance for reasonable predictions. So far, so good, unless you have recognized the x-part of the equation I just mounted, namely the pattern-concept. It is, of course, not possible to extrapolate analytically determined chemical characters until the patterns of chemical evolution are enunciated, until the chemical phenomena which accompany development of lineages become known.

We now know about patterns of macromolecular evolution and appreciate that nucleic acid and protein data are the best, if not the sole, unifying themes bridging the diversity of organisms (Ferguson 1980). But can micromolecules be used in a similar way? The answer to this question is no unless we are able to provide answers to two relevant problems.

Problems of Micromolecular Systematics

The first problem concerns doubts about the direction of advancement of chemical characters (Harborne 1977a, Harborne et al. 1976). These characters, such as A to E in Fig. 1.2, are elaborated in organisms through se-



quences of reaction steps which one is usually able to formulate. Now, if a taxon contains C, one will not be able to say if it evolved from a taxon accumulating B (by expansion of the reaction sequence), from a taxon accumulating D (by reduction of the reaction sequence) or from a taxon accumulating E (by concomitant blocking of the pathway leading to E and enhancement of the pathway B → D). This, however, is essential knowledge and it will never be possible to use micromolecular characters as a basis for evolutionary classifications until it becomes clear when and where to expect expansion, reduction or replacement of a reaction sequence; until some basic principles foretell the direction in which an evolutionary series has to be read.

The second problem concerns doubts about how to unify the messages contained in all the different biogenetic groups of micromolecules. To quote examples from our own work only, allyl- and propenylphenols (Gomes et al. 1978), neolignans (Gottlieb and Kubitzki 1981a,b), coumarins (Silva and Gottlieb 1981), flavonoids (Fernandes et al. 1978, Gomes et al. 1981a), isoflavonoids (Albuquerque et al. 1981, Cagnin and Gottlieb 1978, Gomes et al. 1981b, Oliveira et al. 1971), neoflavonoids (Oliveira et al. 1971), pyrones (Gottlieb 1972, Gottlieb and Kubitzki 1981a,b), xanthones (Kubitzki et al. 1978, Rezende and Gottlieb 1973), benzylisoquinoline alkaloids (Ferreira et al. 1980, Rezende et al. 1975), quinoline alkaloids (Silva and Gottlieb 1981), quinolizidine alkaloids (Salatino and Gottlieb 1980, 1981a,b), indolo-iridoid alkaloids (Cagnin et al. 1977), iridoids (Kaplan and Gottlieb 1982), polyacetylenes (Ferreira and Gottlieb 1982) and non-protein amino acids (Gomes et al. 1981a) are usually employed as systematic markers for particular plant taxa. It is shown in this book, however, that different groups rarely accumulate, i.e. are subject to structural variation, in the same species or group of species and that, indeed one group of metabolites may replace another in morphologically related taxa. If we accept this as evidence that these different groups of metabolites perform analogous functions, say defence or attraction of pollinators, we may find a common characteristic capable of measuring evolutionary advancement of a metabolite irrespective of the biogenetic group to which the metabolite belongs. This means, of course, that we will have to shift emphasis from the structure of accumulated

molecules to alterations of biosynthetic pathways. Although this requirement was clearly stated years ago (Birch 1963), its recognition was not immediately helpful. No procedures existed to assess the significance of such alterations in connection with plant evolution (Birch 1973).

The natural products chemist knows of only one way to deduce biosynthetic correlations: inspect the structure of micromolecules and analyse their common features in relation to natural occurrence, a task which discloses several conspicuous trends of micromolecular evolution. Interpreted in terms of basic principles they may prove of value in elevating micromolecular systematics from an old art into a scientific discipline.

Principles of Micromolecular Systematics

Is it biosynthetic diversification or is it biosynthetic simplification of chemical structure which runs parallel with evolution? The answer to this old problem of micromolecular systematics is: it is both. The difference is one of intensity. Evolution on a higher systematic level is accompanied by contraction of reaction steps and diversification. This is reflected in the expansion of reaction steps and diversification. These two trends are depicted in two principles of micromolecular systematics which are derived from the integration of a broad data base (Figs. 4-14) for different compound groups occurring in the plant kingdom.

The first principle (Fig. 2.1, top) states that evolution of the primary precursor (from which biogenic groups of secondary metabolites are derived) proceeds by blocking reaction steps. Such blocking leads to new

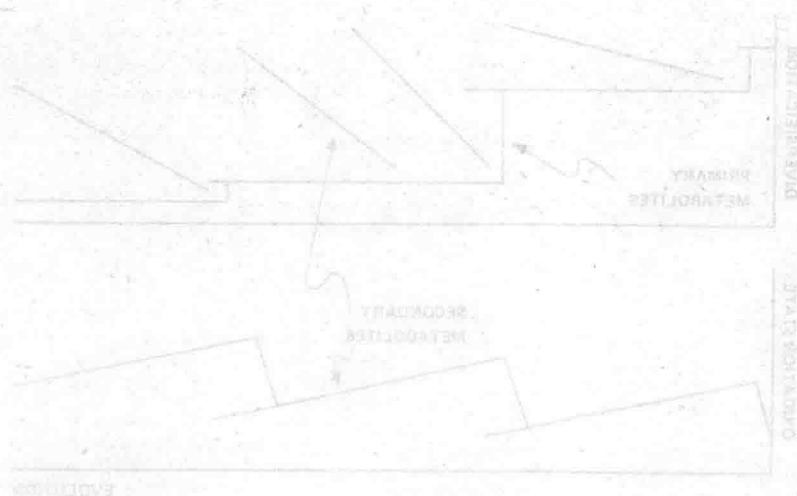


Fig. 2.1. Basic concepts of micromolecular evolution

CHAPTER 2

Postulates of Micromolecular Evolution

Principles of Micromolecular Systematics

Is it biosynthetic diversification or is it biosynthetic simplification of chemical structure which runs parallel with evolution? The answer to this old problem of micromolecular systematics is: it is both. The difference is one of hierarchy. Evolution on a higher systematic level is accompanied by contraction of reaction steps and oxidations and on a lower level by expansion of reaction steps and deoxygenations. These postulates are embodied in two principles of micromolecular systematics which, together with two additional principles, are derived from the interpretation of a broad data base (Chaps. 4–14) for different biogenetic groups diversified in the major plant taxa.

The first principle (Fig. 2.1, top) states that evolution of the primary precursors (from which biogenetic groups of secondary metabolites are derived) proceeds by blocking reactions steps. Such blocking leads to new

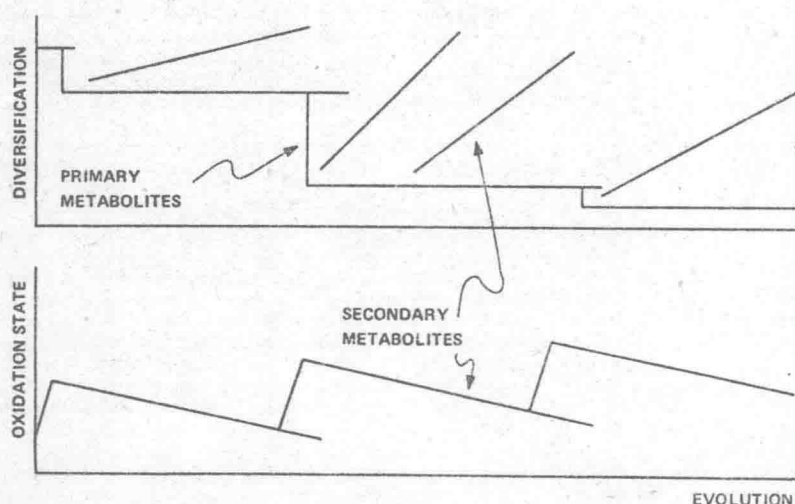


Fig. 2.1. Basic concepts of micromolecular evolution

chemical lines. Within each line evolution of the metabolites belonging to a biogenetic group proceeds by diversification.¹ The second principle (Fig. 2.1, bottom) states that evolution of micromolecules proceeds by oxidation. The relatively highly oxidized compounds characterize new chemical lines. Within each line evolution proceeds by deoxygenation.

Although evolution can thus be rationalized by separate consideration of primary and secondary metabolism, such a distinction is somewhat artificial. Indeed, the use of single biogenetic groups for classificatory purposes will at best lead to partial results.

The precursors of all biogenetic groups are of course present in all plants where they provide essential macromolecules. It is thus plausible that the occurrence of identical chemical substances is largely due to parallelism. Although compounds may appear scattered throughout the plant kingdom, only if secondary metabolites are accumulated in certain plant groups will they serve a useful purpose in biochemical systematics. It may appear, *a priori*, that distinction between haphazardly distributed metabolites and specially accumulated metabolites would be difficult or even impossible (Hegnauer 1976). This is not the case, since accumulation of secondary metabolites from the chemosystematic standpoint refers not merely to qualitative or quantitative aspects, but chiefly to versatility of structural variation on a biosynthetic theme.

Members of the same species usually contain, against a background of chemosystematically irrelevant metabolites (unless considered in relation to ancestry), only a limited number of relevant biogenetic groups of micromolecules. Different groups, supposedly with analogous functions, may nevertheless be produced by closely related plant taxa, possibly either in response to environmental pressure or due to metabolic limitations. Enhancement of products along one biosynthetic route seems to trigger a regulating mechanism which blocks the formation of homologous compounds along another.

From the standpoint of biochemical systematics, these arguments are embodied in the third principle: the homology of biosynthetic routes, not the substances produced, is a plausible indication of phylogeny (Brand 1978); or while resemblance of taxa with respect to structural variation of the metabolites of their chemosystematically meaningful biogenetic group is a reasonable indication of affinity, differences in the chemical compositions of taxa say nothing about their lack of affinity.

So far we have been concerned exclusively with micromolecular evolution leading to allelochemicals. Clearly, however, this cannot be dissociated

¹ This second part of the first principle was formerly designated second principle (Gottlieb 1980b)