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# THE RATIONAL SEARCH FOR NATURAL NEOLIGNANS

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*A rational method of search for natural neolignans of desired structures is outlined. This involves consultation of a collection of chemical profiles of plant families. The profiles are assembled considering the biosynthetic class (in the present case lignoids), subclass (neolignans), structural types (neolignan skeleta) and relative frequency of substitutional derivatives belonging to each type (known compounds). The method is of course applicable to any class of natural products. Its use in the case of neolignans is here selected as an example in view of the recently discovered antagonism towards PAF of kadsurenone, a representative of this subclass of phytochemicals. Application of the chemical profiles to phylogenetic studies is illustrated.*

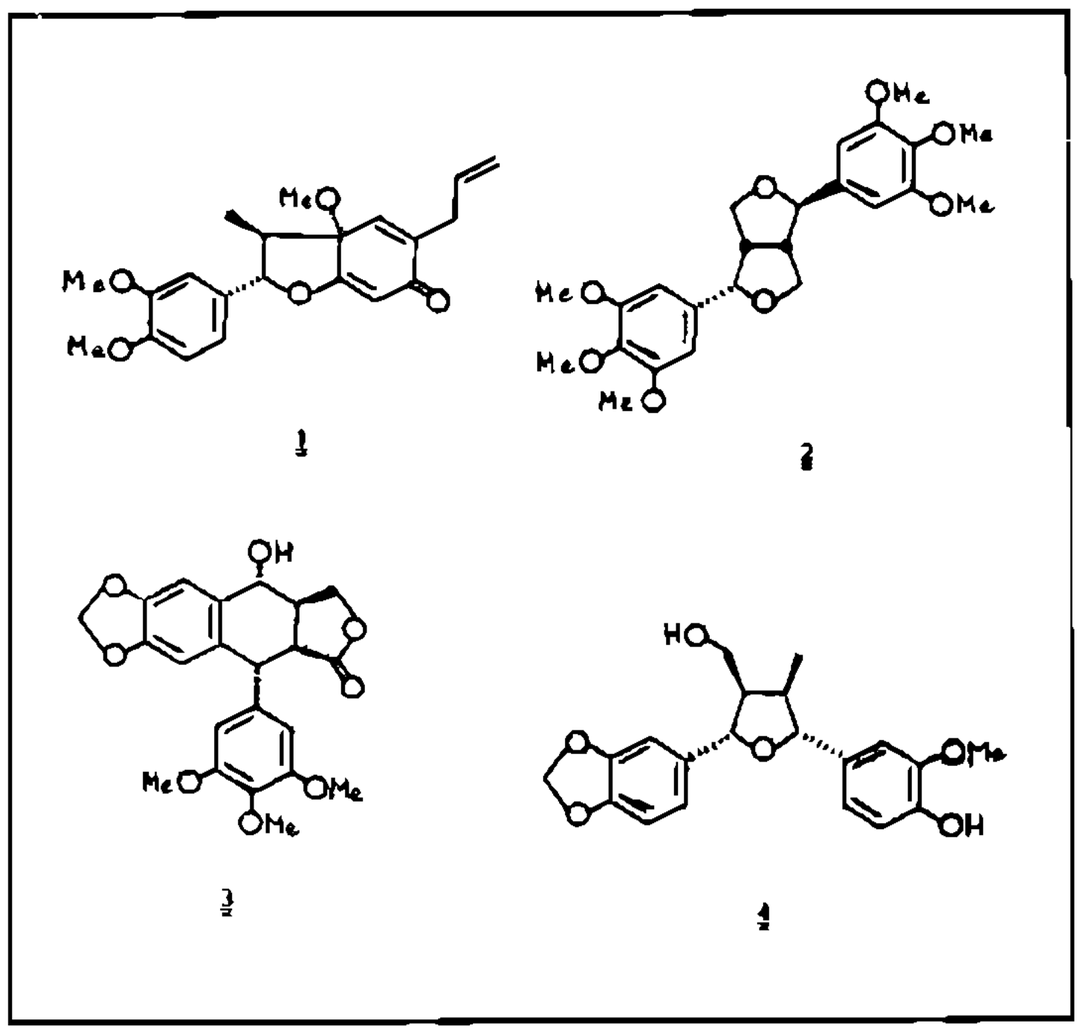
Key words: neolignans – natural products – chemosystematics – chemical profiles – rational search

Recently Shen et al. (1985) reported that the neolignan kadsurenone (1) is a potent antagonist of PAF, the platelet aggregation factor, mediator of inflammatory diseases. When the same group (Chang et al., 1985) disclosed later that *Piper futokadsura*, where-from 1 had been extracted, contained other equally active structural analogues, it seemed probable that still other neolignans, potentially endowed with bioactivity of different potency and/or type, may be produced by plants. Traditionally the search for specific phyto-chemicals involves experimentation by trial and error. However, considering the wealth of available data, at present this cumbersome and time consuming procedure can frequently already be replaced by a more rational approach (Kaplan & Gottlieb, 1990). In the specific case a significant number of reports on neolignans is indeed available (Gottlieb & Yoshida, 1989) and it is the purpose of the present paper to demonstrate, by application to the kadsurenone problem, a method which may prove of general usefulness.

The method consists of several successive steps: 1. Selection of the biosynthetic class or subclass to which the particular plant metabolite belongs. 2. Establishment, within the bio-synthetic class, of metabolic sequences leading to structural types. 3. Determination, within the structural types, of the relative frequency of the included compounds with respect to all compounds of the biosynthetic class in each plant family. 4. Construction, using the data obtained in items 2 and 3, of the chemical profile of a plant family (this can be done only for one biosynthetic class or subclass of com-pounds, as shown here, or for several classes or subclasses).

## RESULTS

Kadsurenone (1) has a $(C_6C_3)_n$ formula and thus is a lignoid. Representatives of this class of natural products in which, as in kadsurenone, n = 2, are designated lignans (e.g. 2 and 3) and neolignans (e.g. 1 and 4). The difference between these subclasses concerns, rather than structural features, the presence (lignans) *versus* absence (neolignans) of oxygen functions at the terminal carbons of the $C_3$-side chains. This difference in functionality is suggested to be due to the derivation from four distinct biosynthetic precursors (Fig. 1, line 1): at least one cinnamyl alcohol unit *plus* another such unit or, less commonly, a cinnamic acid unit (lignans, e.g. respectively 2 and 3), *versus* at least one propenylphenol (or allylphenol) unit *plus* another such unit or less commonly, a cinnamyl alcohol unit (neolignans, e.g. respect-ively 1 and 4). These four types of precursors are intermediates of the shikimate pathway (Gottlieb, 1989), thereon being separated by two important energy barriers (Fig. 1, line 1). Indeed, reductive power and its specific asso-ciated enzymatic machinery are needed first to transform the carboxyl of cinnamic acids via the formyl group to the hydroxymethyl of cinnamyl alcohols and next to eliminate the




hydroxyl either by a direct nucleophilic displacement to propenylphenols or by an allylic one to allyphenols. Significantly, the first reduction proceeds with all natural substrates, inclusively with cinnamic acid itself, whereas the natural existence of propenylbenzene and allylbenzene has never been observed. Hydride displacement of the hydroxyl from cinnamyl alcohols requires the assistance of a conjugated electron donor, such as an *ortho* or *para* situated phenolic hydroxyl. The aromatic substitution of the compounds represented in Fig. 1 thus represents a minimum requirement. In specific cases additional oxy-functions may occur.

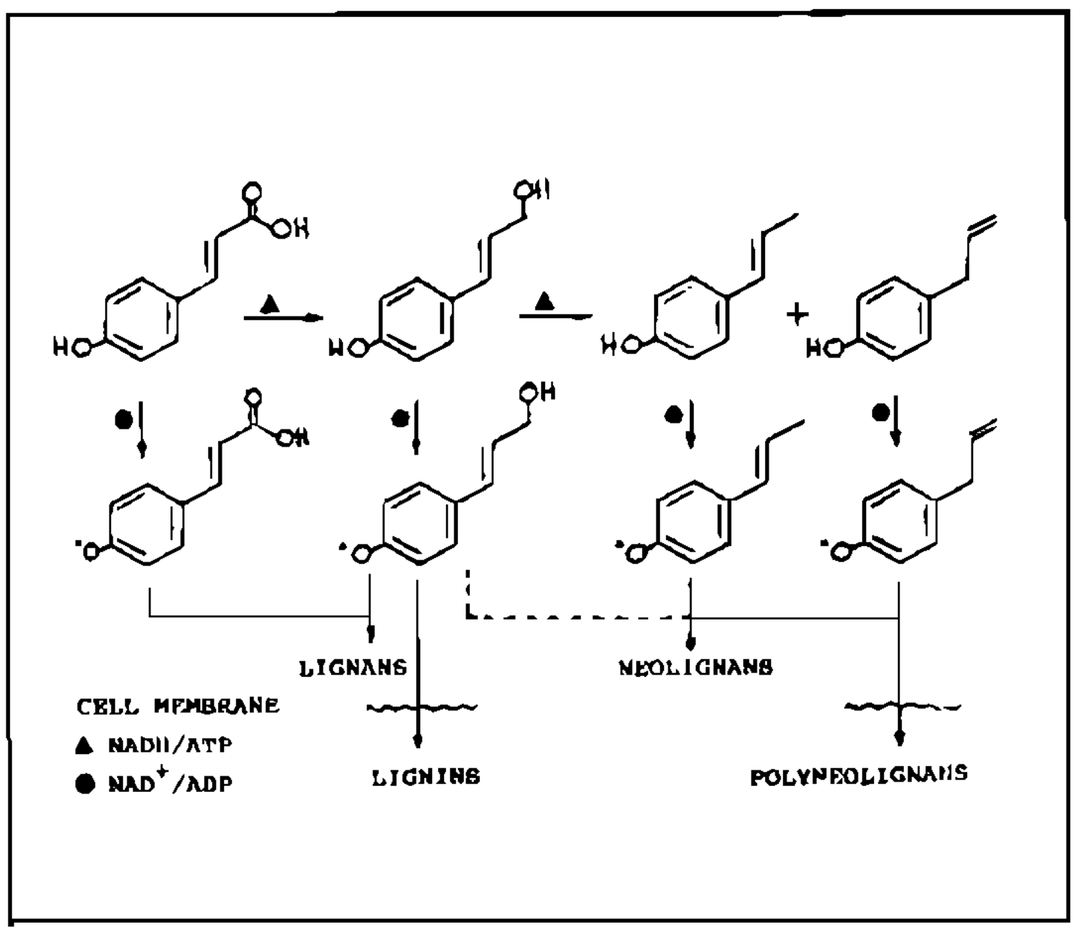



Fig. 1: schematic representation of two reductive steps added to the shikimate pathway, the first one with lignification of land plants, ca. 400 million years ago, and the second one with flowering plants, ca. 100 million years ago, which justify the oxidative production of cellular lignans and neolignans and extracellular lignins and polyneolignans.

Both pairs of precursor species, cinnamic acids and cinnamyl alcohols as well as propenylphenols and allylphenols, can be oxidatively transformed into phenoxide radicals (Fig. 1, line 2). A lower number of conjugated positions for the unpaired electron (Fig. 2) explains the higher resistance against oxidation by allylphenol, as expressed by their quantitative and qualitative predominance in comparison with propenylphenols. Still within the cell, i.e. in contact with an enzyme surface, such radicals give chiral dimers, respectively lignans and neolignans (Fig. 1, line 3). Even if some trimers or tetramers may also be formed by this reaction, there must be clearly a limit to the oligomerization imposed by, again template requiring, protection of oxidizable phenolic hydroxyls by O-methylation and O,O-methylenation reactions. Polymerization respectively to lignins and polyneolignans (the latter as yet hypothetical entities) can take place only after transport of the enzymatically generated radicals across the cell wall, again an energy dependent process (Fig. 1, line 4).

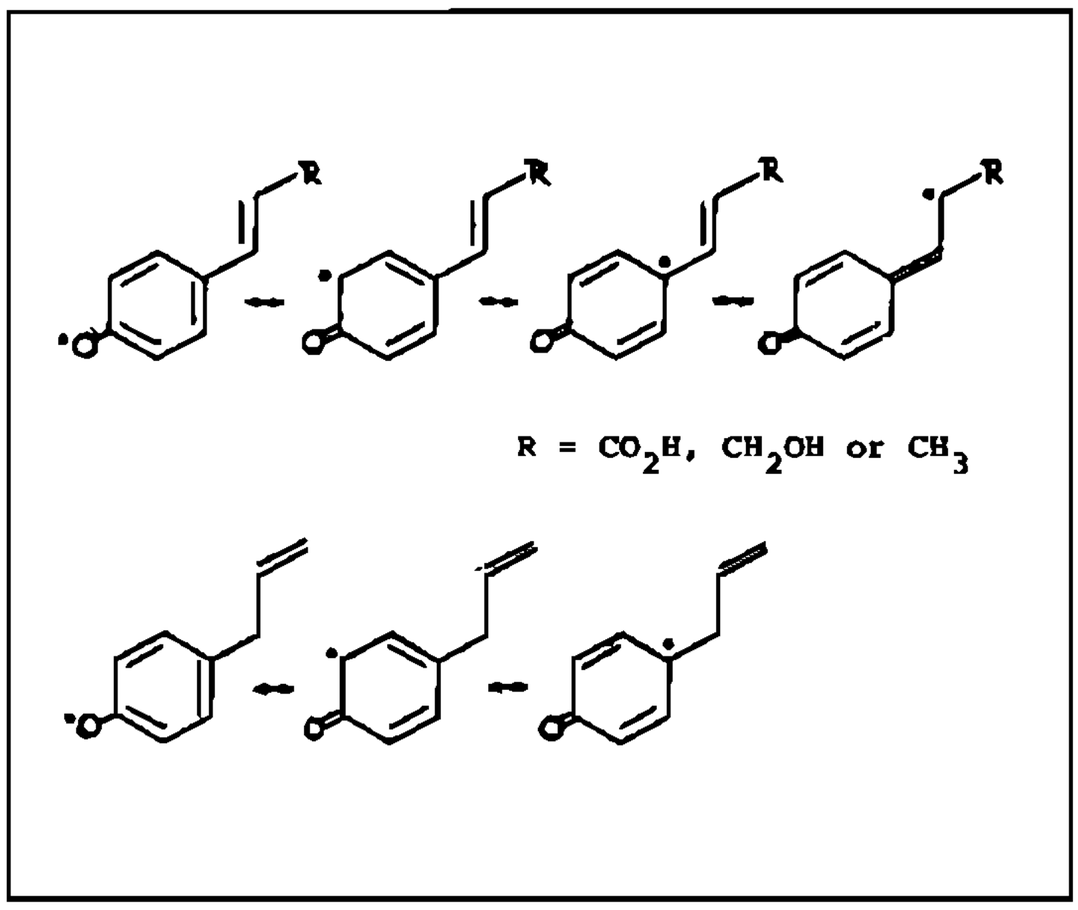



Fig. 2: schematic representation of the mesomeric forms of the phenoxide radicals shown in Fig. 1.

Thus lignins are formed in intercellular space, i.e. over cellulose matrixes, reason why none of their degradation products obtained so far has shown optical activity. An even more convincing structural evidence that their formation does not involve oligomeric cellular building blocks is seen in the fact that, in contrast to lignans and neolignans, lignins have never been found to bear methylenedioxy

substituents. Laboratory experiments have shown that the oxidation of guaiacyl units into piperonyl units proceeds only if a large substituent forces the methoxyl carbon towards the hydroxyl (Fig. 3, lines 1 and 2), an arrangement which in nature, in absence of such a substituent, can be supposed to depend on enzymatic steering (Fig. 3, line 3).

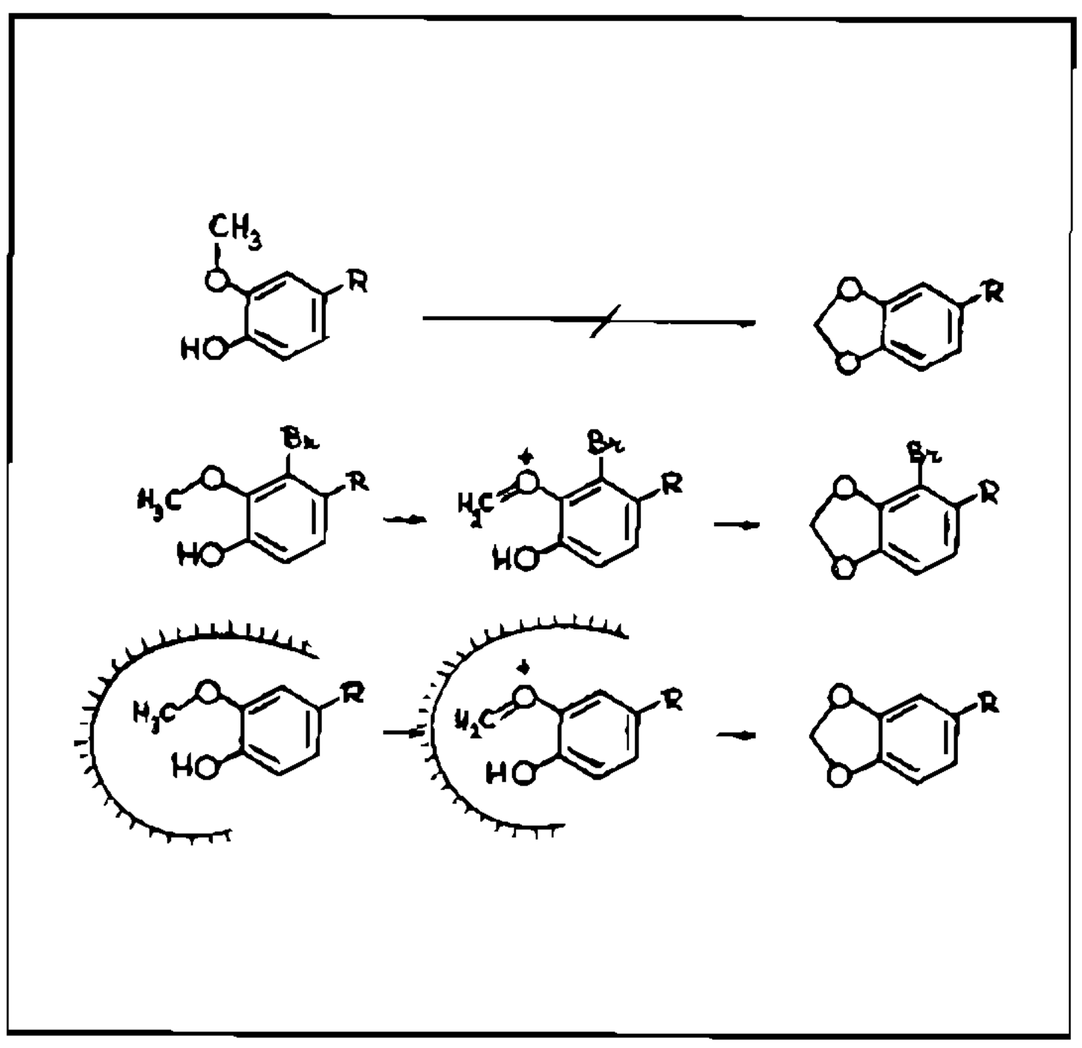



Fig. 3: oxidative formation of the methylenedioxy group *in vitro* (line 2) and *in vivo* (line 3).

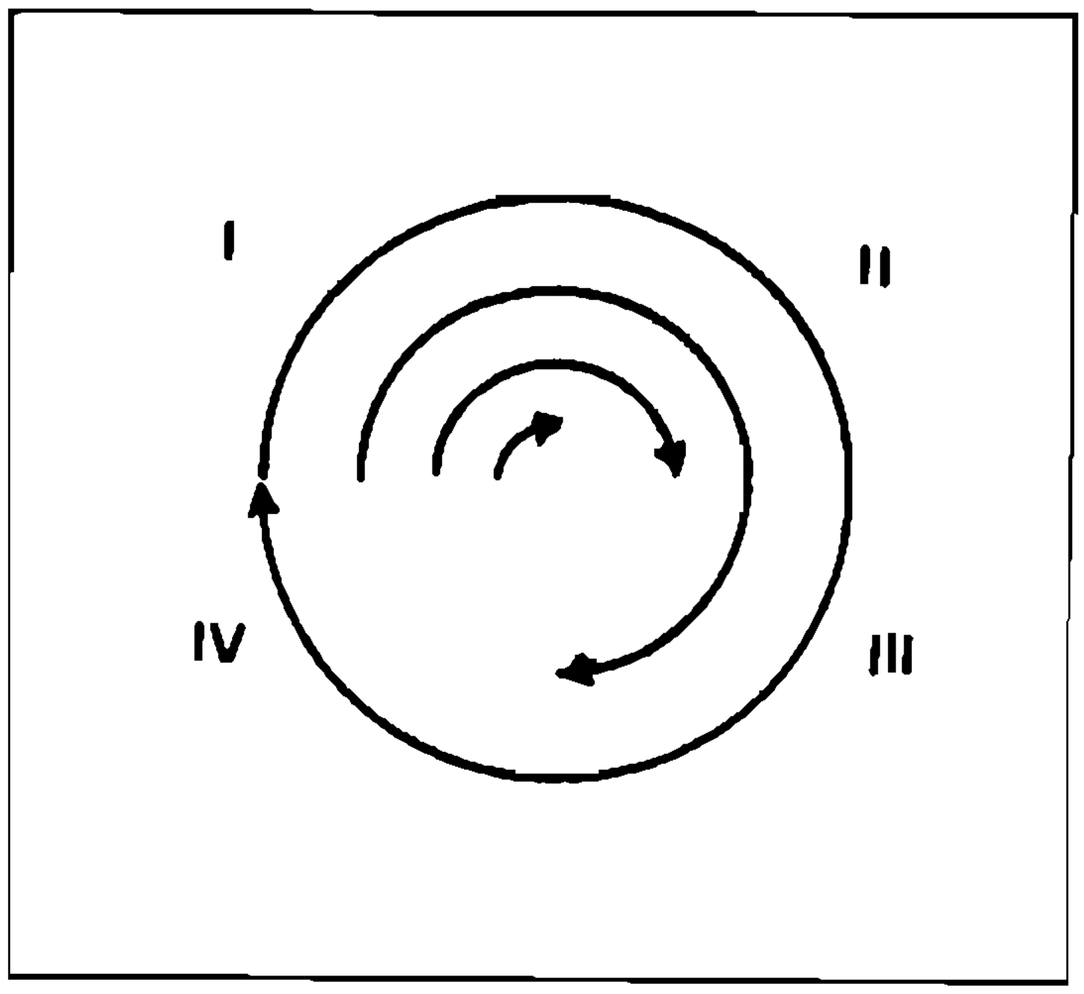



Fig. 4: representation of distributional trends of lignoids in dicotyledons. The spiral arrangement indicates the progressively diminishing importance of diversification or accumulation of neolignans (NLG), lignins (LIG) and lignans (LGN), in this order, upon passing from plant groups symbolized respectively by I (e.g. Magnoliiflorae), II (e.g. Rosiflorae) and III (e.g. Asteriflorae) towards group IV (e.g. Gentianiflorae and Lamiiflorae) characterized predominantly by cinnamic acids (CA). Cinnamic acids are here considered to include not only free acids (Molgaard, 1985), but also derivatives such as flavonoids. Designations of superorders *sensu* Dahlgren (1980).

An approximate sequence for the highest to lowest energy requirement in the formation of lignoid subclasses might read as follows: Polyneolignans, neolignans, lignins, lignans, cinnamic acids. In primitive angiosperms the shikimate pathway has attained its climactic development and hence, for reasons of economy, the evolutionary abandonment of these subclasses might operate in the same order. Indeed, cinnamic acids and lignans are the most conspicuous lignoids in the most advanced angiosperms. In the more primitive members their presence is superimposed on, and possibly even repressed by, the formation of polyneolignans and neolignans (Fig. 4). This trend towards the gradual elimination of lignoids places lignins at an intermediate position. They lose importance in direction of the more highly evolved groups and indeed the most easily appreciated evolutionary trend in the gross morphology of flowering plants concerns the successive appearance of trees, shrubs, herbs and weeds.

Hence in general outline the kadsurenone problem is solved. Diversification of the neolignan theme occurs in primitive, woody angiosperms and it would clearly be most profitable to look into Magnoliiflorae and their close relatives for additional compounds of this lignoid subclass. However, if it is desired to obtain information also concerning the most likely source of specific structural variants, a classification of neolignans must first be constructed. According to our proposal, this considers, as primary criterion, their biogenetic origin by oxidative coupling of radicals derived from propenylphenols + propenylphenols, propenylphenols + allylphenols and allylphenols + allylphenols. Coupling within such pairs of precursor radicals across particular skeletal positions (for numbering of positions see e.g. 1 and Fig. 2) leads for these three neolignan groups respectively to seven (designated A to G), five (designated M to Q) and five (designated V to Z) structural types. Eventual couplings across additional positions are of course not excluded. The relative frequency of these

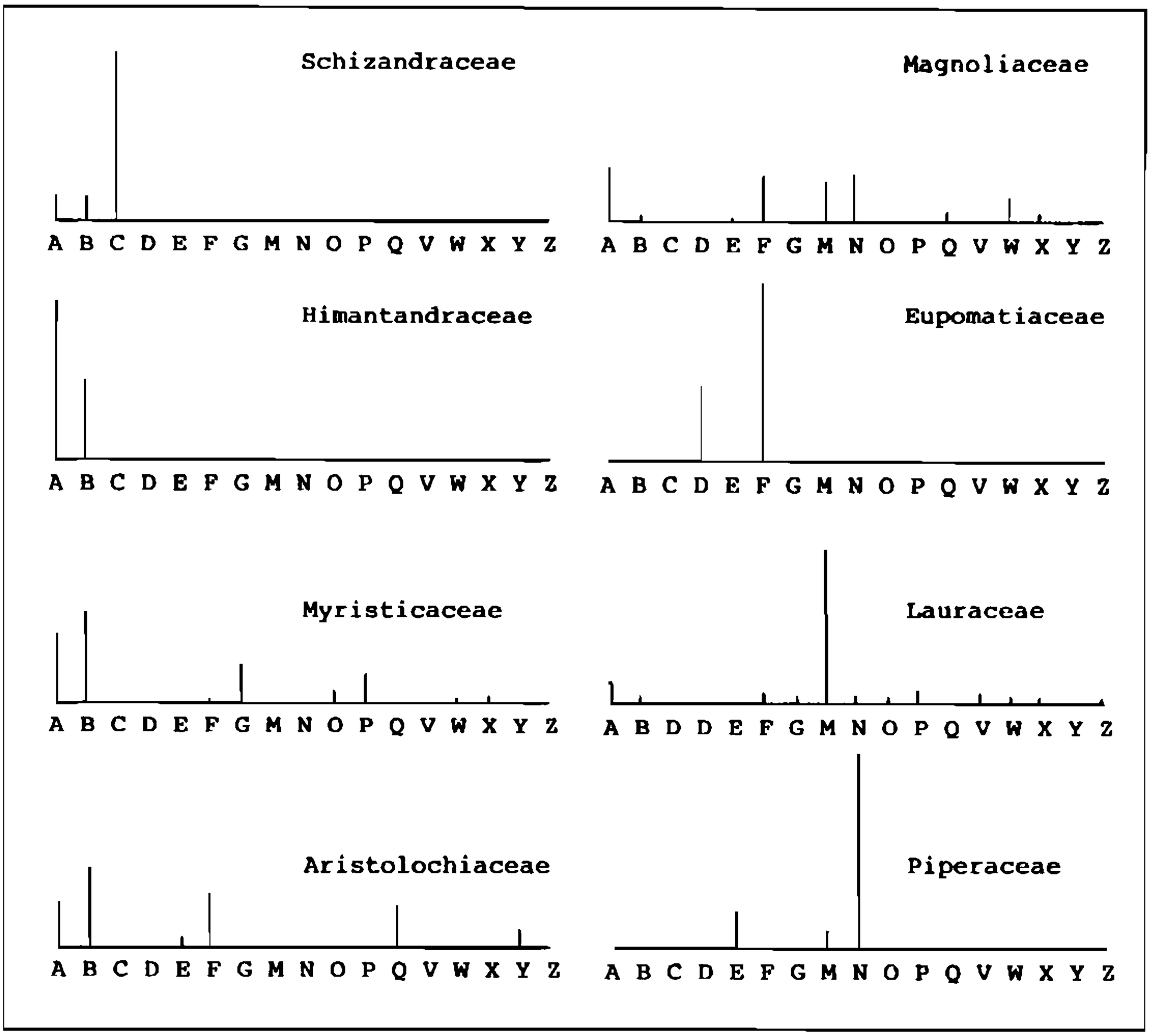



Fig. 5: neolignan profiles (percentage frequency of neolignans) in angiosperm families. Fundamental coupling of radicals, *ex* propenylphenols + propenylphenols: A 8.8'; B 8.8',6(2).7'; C 8.8',2.2'; D 8.8',2.1'; E 8.8',7.7'; F 8.5'; G 8.O.4'; *ex* propenylphenol + allylphenol: M 8.1'; N 8.3'; O 8.5'; P 8.O.4'; Q 8.7'; *ex* allylphenol + allylphenol: V 7.1'; W 5.5'; X 4.O.5'; Y 1.5'; Z 2.O.3'.

neolignans *per* type in families of the magnolialean block (Fig. 5) indicate that, considering presently available data, with respect to kadsurenone the chances of finding derivatives belonging to the same type N outside the Piperaceae are best for Magnoliaceae and Lauraceae.

## DISCUSSION

The neolignan profiles for families suggest furthermore in the magnolialean block Magnoliaceae to constitute a basic group which developed into two major lineages, characterized respectively by the diversification of types A and B (Himantandraceae, Myristicaceae, Aristolochiaceae) and of types M and N (Lauraceae, Piperaceae). Schizandraceae and Eupomatiaceae appear to be highly modified members respectively of the former and the latter lineages. Chiefly Schizandraceae are important on account of the antihepatotoxic properties of their dibenzocyclooctane neolignans. Nevertheless, as shown by their neolignan profiles, the chances of localizing such compounds of type C in other families of the magnolialean block are quite low. Surprisingly one or two additional compounds of this type have been located in the completely unrelated, and highly advanced, Verbenaceae.

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