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THE ROLE OF OXYGEN IN PHYTOCHEMICAL EVOLUTION TOWARDS DIVERSITY*

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Key Word Index—Secondary metabolites, micromolecules, diversity, evolution; ecology; skeletal specialization; oxidation level, biosynthetic pathways, atmospheric composition, molecular stabilization; biosynthetic flexibility.

Abstract—Methods have recently been proposed for the quantitative correlation of morphological and micromolecular traits of plants. Their application to plant groups of high hierarchic rank shows morphological evolution to be accompanied by progressive oxidation of secondary metabolites within particular biosynthetic classes. In angiosperms the evolutionary replacement of woody by herbaceous forms is accompanied by the gradual curtailment of the shikimate pathway for the production of secondary metabolites and the concomitant enhancement of the acetate and mevalonate pathways for the synthesis of polyketides and terpenoids of high oxidation level. These results suggest that micromolecular evolution is dependent on the variable oxygen content of the atmosphere However, oxygen does not act directly on micromolecular evolution, but triggers the evolution of enzymatic protective devices, such as selective etherification, Schiff base formation or reduction for the regulation of the half lives during the oxidative turnover and for the orientation of the biosynthetic pathways of micromolecules. Both phenomena are essential in the diversification of secondary metabolites and hence requisites for the flexibility of an organism's adaptation to the environment

INTRODUCTION

A molecular definition of life (Fig. 1) [1] shows the biosynthetic path from the macromolecules of the genotype to the macromolecular assemblies of form and the micromolecules, so called secondary metabolites, of the phenotype often associated with natural selection. Be it for taxonomic purposes, in quest of biodynamic products or in the hope of understanding nature, since the beginning of scientific classification man has endeavoured to compare morphology and molecules. However, in the absence of methods for the quantitative correlation of these characteristics the results were necessarily descriptive Recently Sporne calculated percentage indices (SI) to portray the relative morphological advancement of angiosperm families [2]. It is hoped that such statistical data will in due time become available for all plant groups and on all hierarchic ranks [3]. Meanwhile we developed comparable advancement indices based on the extraction of appropriate information from structural formulae of micromolecules.

METHODS

In our work up to 1982 [1], compounds were characterized by a parameter involving biogenetic pathways (S) and by oxidation level (O) While the O-values were then and continue to be easily accessible, the S-values were difficult to produce, often requiring hypothetical decisions on biosynthetic sequences and tedious determinations of the frequency of a compound's occurrence. Because of this last requirement, the S-values were temporary and had to be re-calculated with each fresh

*The late Professor Tony Swain wrote on one of my manuscripts 'keep it up' I did, and wish to dedicate the present demonstration of continuing effort to his memory

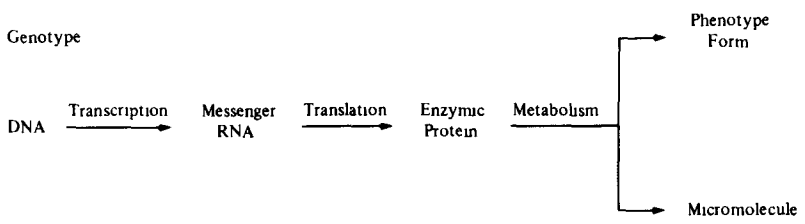

Fig. 1

report on the compound. In all our more recent papers [4–11], the determination of skeletal specialization (S) requires only the recognition of the compound's biogenetic affiliation, followed by a simple calculation independent of prior or posterior registries of occurrence, as described in the next paragraph and exemplified by limonoids (Fig. 2, Table 1)

The skeletal specialization (S) of a compound (per carbon, C) with respect to the general precursor of its biosynthetic class is determined by counting the number of bonds (to C) broken and the number of bonds (to C, or to a heteroatom if this involves formation of a new cycle) formed for each carbon of the compound; the total counts obtained are then divided by the number of C-atoms in the compound The oxidation state (O) of a compound (again per carbon) is determined by counting, for each carbon of the compound, -1 for each bond to H and $+1$ for each bond to a heteroatom, again these counts are added and divided by the number of C-atoms of the compound Loss of a C-group is considered to operate through a carboxylated intermediate and for each severed C–C bond which results in the loss of a molecular unit (in comparison with the precursor) three points are added to the count Implicit in this description is the possibility of limiting the calculation of S- and O-values to selected molecular moieties or of deducing other parameters involving phenomena such as glycosylation (Gl) and methylation (Me)

A plant species may contain several compounds of the particular biosynthetic class, each characterized by S-, O-, Gl- and Me-values The averages of these values are considered to represent the evolutionary advancement parameters, respectively EA_S, EA_O, EA_{Gl} and EA_{Me}, of the species with respect to biosynthetic class The averages of the EA parameters for the species of their genus are considered to represent the EA parameters of that genus The averages of the EA parameters for the genera of their family are considered to represent the EA parameter of that family, and so on up to the desired hierarchical rank

In special cases, the application of specific methods may be more convenient Thus 'S'-values for polyacetylenes are considered to be equal to their numbers of carbon atoms These decrease from 18 (in the precursor types) to 8 (in the most highly degraded, i e specialized, compound type) [12] Thus 'O'-values for lignins are determined by multiplying the percentage of *p*-hydroxyphenyl, guaiacyl and syringyl components by the number of oxygen atoms, respectively 1, 2 and 3, linked to their aryl moieties and adding the products Analogously 'O'-values for flavonoid A-rings are determined by multiplying the percentage of 5,7-dihydroxylated, of 5,6,7- and 5,7,8-trihydroxylated and of 5,6,7,8-tetrahydroxylated components respectively by 1, 2 and 3 and adding the products.

It is often desirable to characterize a taxon by the number of compounds (NC) belonging to a selected

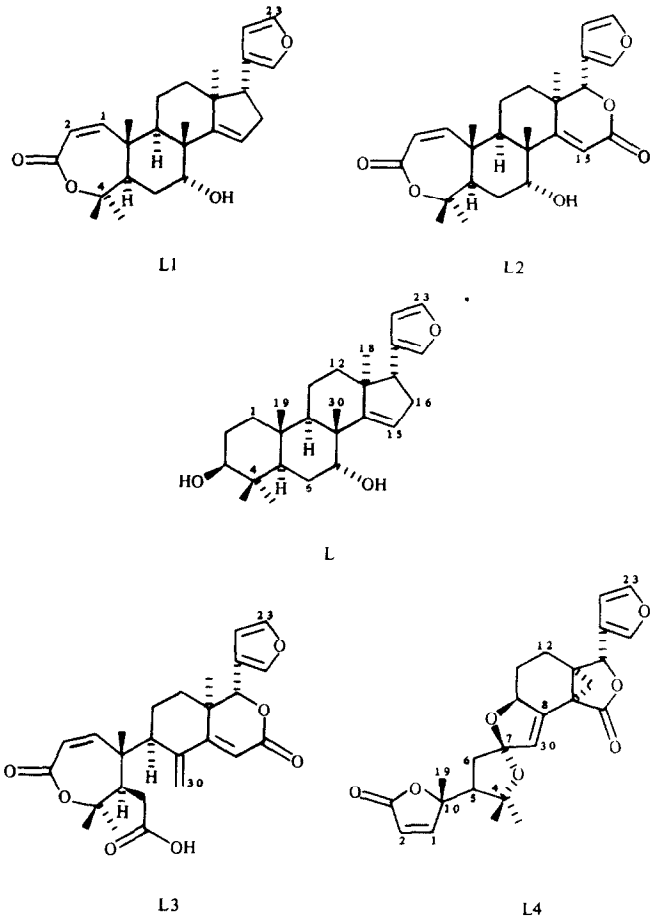

Fig 2

Table 1 Determination of skeletal specializations and oxidation levels of the selected limonoids L1, L2, L3 and L4 in relation to L, the biosynthetic precursor (for structures and numbering of carbons see Fig 2)

	L	L1	L2	L3	L4
Total number of carbons	26	26	26	26	25
Position of carbons with broken bonds		3, 4	3, 4, 16, 17	3, 4, 7, 8, 16, 17	3, 4, 7, 8, 9, 10, 15, 16, 16, 17
Position of carbons with new bonds		3, 4	3, 4, 16, 17	3, 4, 16, 17	3, 4, 7, 7, 7, 9, 10, 14, 15, 17, 18, 30
Number of modified carbons	0	4	8	10	22
Skeletal specialization (S)	0/26=0	4/26=0 15	8/26=0.31	10/26=0 38	20/25=0 88
Number of C–H bonds	36	33	31	29	28
Number of C–O bonds	4	7	11	13	17
Sum of -(C–H)+(C–O) bonds	−32	−26	−20	−16	−11
Oxidation level	−32/26=−1 23	−26/26=−1.00	−20/26=−0.77	−16/26=−0.62	−11/25=−0.44

biosynthetic class [1, 13]. If several such classes occur, the relative importance (RI) of each can be characterized by the number of compound types (NT) and the number of compounds (NC): RI = NT × NC [11].

To conclude this section on the extraction of chemosystematic information from structural formulae a comment is in order: mean values of a numerical parameter are less prone to change with the incorporation of new data into the system, and thus produce more stable and meaningful results rather than extreme values. If it is desired to use extreme values at all, it is best to convey the spread of values from the lowest to the highest with indication of the frequency of occurrence [Emerenciano, V. de P., Ferreira, Z. S. and Gottlieb, O.R., unpublished results].

In addition to Sporne type indices concerning morphological advancement of families, similar devices may be of use. Thus woodiness can be calculated by the expression [Σ (habit of genus × number of species of genus)]/[number of species of family]. Habits of genera are assigned values 1, 25, 50, 75 and 100, according to indications of standard references with respect to herbs, herbs or shrubs, shrubs, shrubs or trees, trees. Intermediate values, such as 62.5 for trees predominantly shrubs can also be used. Herbaceous form ('herbacity') = 100 − woodiness [1].

RESULTS

Micromolecular evolution within biosynthetic classes

The application of evolutionary advancement parameters (EA) respectively to skeletal specialization (S) and oxidation level (O) of carotenoids in different algal families shows that EA_s and EA_o values are positively correlated (Fig. 3) [Teixeira, V. L., Kelecom, A. and Gottlieb, O. R., unpublished results] In addition, pigment associations are seen to be selective: carotenoids of relatively low and high mean S and O values co-occur respectively with chlorophylls *a* and *b* and with chlorophylls *a* and *c*. The plot provides a quantitative dimen-

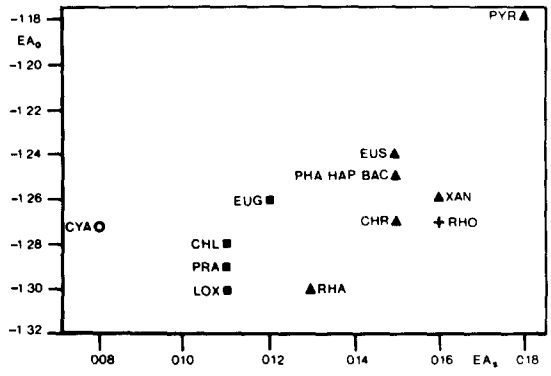

Fig. 3 Correlation of carotenoid based EA_o and EA_s values for algae CYA . . Cyanophyceae, LOX . Loxophyceae, PRA . . Prasinophyceae, CHL . . Chlorophyceae, EUG Euglenophyceae, RHA . . Rhaphidophyceae, CHR . Chrysophyceae, BAC . Bacillariophyceae (Diatomeae), HAP . Haptophyceae, PHA . Phaeophyceae, EUS . Eustigmatophyceae, RHO . . . Rhodophyceae, XAN . Xanthophyceae, PYR . . Pyrrhophyceae (Dinoflagellatae) ○: chlorophyll *a*, phycobilins. △ · chlorophylls *a* and *c*, phycobilins + chlorophylls *a* and *d*, phycobilins ■ . chlorophylls *a* and *b* ▲ chlorophylls *a* and *c*

sion to the proposed evolutionary scheme for thallophytes featuring two branches based on the nature of chlorophylls present [14].

Carbohydrates excepted, lignins are the most abundant and flavonoids are the most widespread materials of terrestrial plants. The shikimate-derived aromatic rings of the former (Fig. 4) and the acetate-derived aromatic rings of the latter (Fig. 5) become somewhat more highly oxygenated upon passing from pteridophytes to gymnosperms and very much more so upon advancing to the more modern angiosperms.

Diterpenoids of several biosynthetic classes are very widespread in vascular plants. For instance numerous labdanes occur in gymnosperms and angiosperms. Labdane based EA_o parameters, calculated by considering O values of compounds × number of compounds per family,

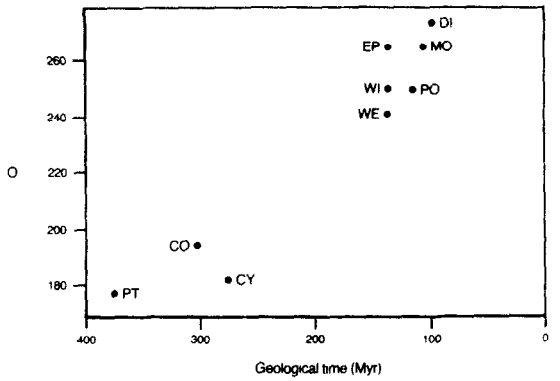

Fig 4. Correlation of 'O'-values (see Methods) of lignins and geological age of the pertinent plant groups PT . Pteridophyta, CO Coniferopsida, CY Cycadopsida, Gnetopsida (WE *Welwitschia*, EP . *Ephedra* & *Gnetum*), PO Poaceae, MO Monocotyledoneae (secondary woody), WI Winteraceae, DI Dicotyledoneae (except Winteraceae) Lignin data and geological ages adapted respectively from refs [15] and [16, 17]

are similar for Gymnospermae, Monocotyledoneae and Rosidae (respectively −1.37, −1.37 and −1.38), but relatively higher for Asteridae (−1.26). A more detailed picture for the two latter subclasses (*sensu* Cronquist [18, 19]) is shown in Fig. 6 obtained without taking into account number of compounds except for the fact that the registry of families with less than four reported compounds is omitted. The plot indicates the existence of a positive correlation of mean oxidation level of labdane-type diterpenes and morphological advance of families. Labdanes have a relatively low skeletal specialization (S = 0.2). For diterpenes of higher S values evolutionary advancement based on oxidation level versus Sporne index (EA_o/SI) correlations for families of Rosidae and Asteridae become blurred. Nevertheless, while minimum EA_o values for families suffer little variation, maximum values become higher with increasing EA_s values, and more importantly in the present context, a trend to higher mean EA_o values is seen to occur upon passing from Dillenidae *plus* Rosidae to Asteridae (Table 2) [Figueiredo, M R., Kaplan, M A C and Gottlieb, O R, unpublished results].

Dahlgren [20] has divided the mainly sympetalous family groups of angiosperms into those which synthesize iridoids and those which synthesize polyacetylenes and sesquiterpene lactones. The former includes the Corniflorae, Loasiflorae, Gentianiflorae and Lamiflorae A positive correlation of the EA_o indices, based on the oxidation values of the reported iridoids, and the mean Sporne indices for the families in each of the orders of these superorders is observed (Fig. 7) [21]

The polyacetylene (-sesquiterpene lactone) groups include the Araliiflorae, the Asteriflorae and some other superorders. Positive correlations of the EA_s and the EA_o indices, based respectively on the skeletal specialization and the oxidation values of the reported polyacetylenes, and the Sporne indices for families of these superorders are observed (Fig. 8) [12]

Correlations of EA_o values based on benzylisoquinoline alkaloids and Sporne indices for families of the Magnoliiflorae and the Ranunculiflorae are also positive (Fig 9) [22]. Interestingly enough the rates of evol-

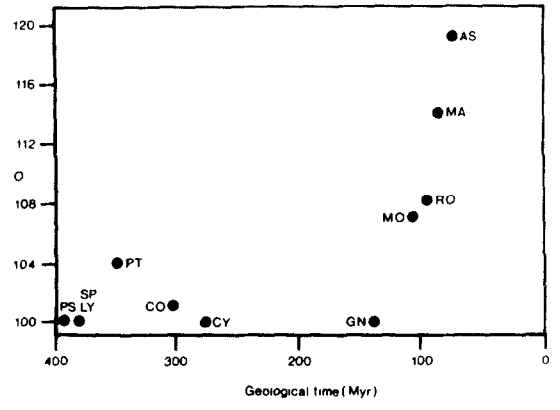

Fig 5 Correlation of 'O'-values (see Methods) of flavonoid A-rings and geological age of the pertinent plant groups PS. . Psilotophytina, LY . Lycophytina, SP Sphenophytina, PT . Pterophytina, CO Coniferopsida, CY Cycadopsida, GN Gnetopsida, MO Monocotyledoneae, RO . Rosidae, MA Magnoliidae, AS Asteridae Flavonoid data and geological ages adapted respectively from refs [1] and [16, 17]

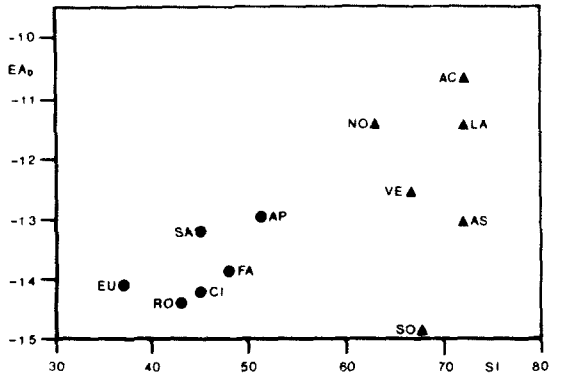

Fig. 6 Correlation of labdane-based EA_o values and Sporne indices for families of the Dillenidae and Rosidae (●) EU Euphorbiaceae, RO Rosaceae, CI Cistaceae, SA Santalaceae, FA Fabaceae, AP Apiaceae, and of the Asteridae (▲) VE Verbenaceae, LA Lamiaceae, AC Acanthaceae, AS Asteraceae, NO Nolanaceae, SO Solanaceae

Table 2. Diterpenoid-based EA_0 values for families of Dilleniidae and Rosidae and of Asteridae

Diterpenoid type	S	Dil. and Ros. families min	mean	max.	Asteridae families min	mean	max
Labdane	0 2	−1 45	−1.38	−1.30	−1 40	−1 24	−1.05
Pimarane	0 3	−1.40	−1 28	−1.10	−1 50	−1.42	−1.35
Abietane	0.5	−1 40	−1.21	−1.10	−1 25	−1.05	−0 90
Kaurane	0 6	−1 30	−1 21	−1.05	−1.25	−1 19	−1.10
Clerodane	0.6	−1.40	−1 07	−0.50	−1 30	−1.00	−0 70

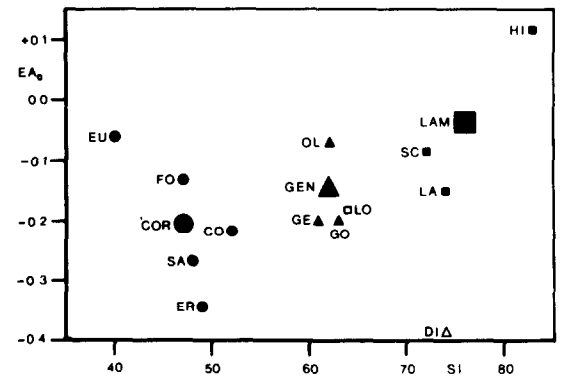

Fig 7. Correlation of iridoid based EA_0 values and mean Sporne indices for families within orders belonging to the superorders CO Corniflorae (●), GEN Gentianiflorae (▲), Loasiflorae (□) and LAM Lamiiflorae (■) *sensu* Dahlgren [20] EU . Eucomiales, FO Fouquierales, SA . . Sarraceniales, ER Ericales, CO Cornales, GE . . Gentianales, OL Oleales, GO Goodeniales, LO . Loasales, SC Scrophulariales, DI Dipsacales, LA . Lamiales, HI Hipuridales

utionary increase in oxidation level is lower for the more primitive Magnoliiflorae than for the more advanced Ranunculiflorae. Besides, the EA_0 values for the former, woody families are uniformly lower than the EA_0-values for the latter, herbaceous families. Thus decreasing woodiness (increasing herbaceous character) of a family runs parallel with increasing mean oxidation level of the alkaloids, and also with increasing number, i.e. diversity, of skeletal types of such alkaloids (Fig. 10) [1]. The phenomenon is accentuated progressively in the families of Magnoliales→Ranunculales→Papaverales, precisely the evolutionary sequence in which these orders are placed by Cronquist [18, 19].

The alkaloid-poor families of the magnolialean complex chiefly diversify neolignans. Both these chemical parameters, as expressed by their numerical relation (in %), versus Sporne indices for each family are correlated in Fig. 11 [13]. The cosmopolitan families Magnoliaceae→Lauraceae→Piperaceae are represented by points which fall along a diagonal. This indicates their possession of both types of constituents, the proportion of neolignans being favoured by evolution. In contrast the geographically restricted families Schizandraceae, Trimeniaceae, Austrobaileyaceae, Eupomatiaceae on one hand and Canellaceae on the other possess respectively neolignans and benzylisoquinolines as replacement characters So far the clearest of these chemical dichot-

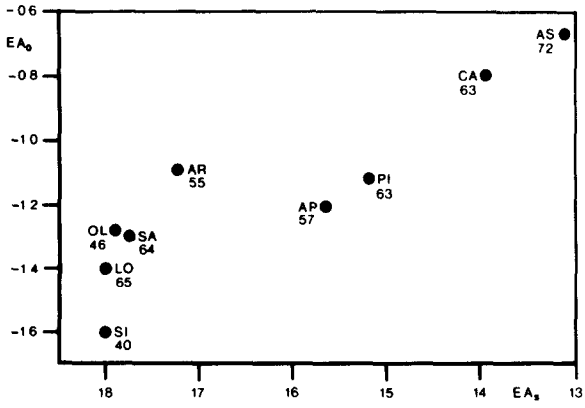

Fig. 8. Correlation of polyacetylene based EA_s (see Methods) and EA_0 values for families of dicotyledons characterized by their Sporne indices [2] Rutiflorae SI . . Simaroubaceae. Santaliflorae LO Loranthaceae, OL . . Olacaceae, SA . . Santalaceae Araliiflorae AR . . . Araliaceae, AP . . . . Apiaceae, PI . . . . Pittosporaceae Asteriflorae . CA . . Campanulaceae, AS . . . Asteraceae. Designations of superorder acc to Dahlgren [20]

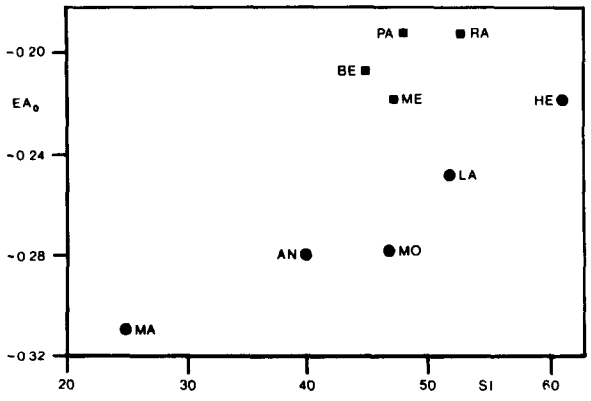

Fig. 9 Correlation of benzylisoquinoline based EA_0 values and Sporne indices for families belonging to the superorders Magnoliiflorae (●) and Ranunculiflorae (■) *sensu* Dahlgren [20]. MA . . . Magnoliaceae, AN . . Annonaceae, MO . . Monimiaceae, LA . . . Lauraceae, HE . . . Hernandiaceae, BE Berberidaceae, ME . . Menispermaceae, RA . . . Ranunculaceae, PA . . Papaveraceae incl Fumariaceae [22].

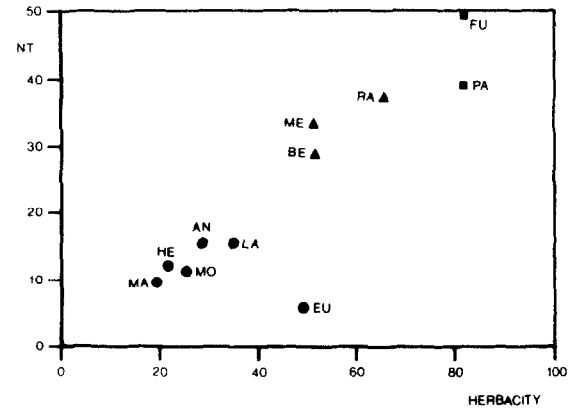

Fig 10 Correlation of 'herbacity' *versus* benzylisoquinoline alkaloid diversity here given by the number of skeletal compound types NT (see Methods) for families of Magnolidae (*sensu* Cronquist [18]) Magnoliales (●) MA Magnoliaceae, MO Monimiaceae, HE Hernandiaceae, AN Annonaceae, LA Lauraceae, EU Eupomatiaceae Ranunculales (▲) BE Berberidaceae, ME Menispermaceae, RA Ranunculaceae Papaverales (■) PA Papaveraceae, FU Fumariaceae [1]

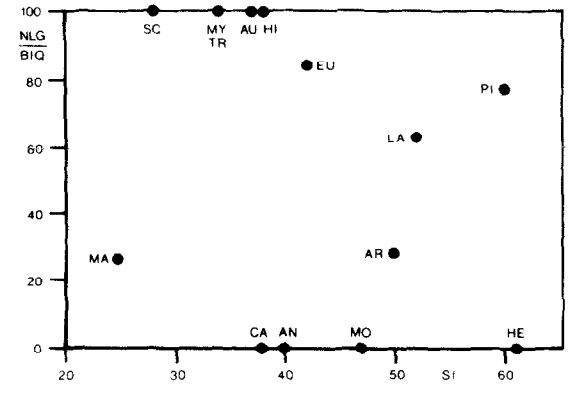

Fig 11 Correlation of the ratio between number of neolignans (NLG) and number of benzylisoquinolines (BIQ), 'NLG/BIQ' = 100 NLG/(NLG + BIQ), and morphological advancement as given by the Sporne Index (SI) in the magnolialean families MA Magnoliaceae, SC Schizandraceae, MY Myristicaceae, TR Trimeniaceae, AU Austrobaileyaceae, HI Himantandraceae, CA Canellaceae, AN Annonaceae, EU Eupomatiaceae, MO Monimiaceae, AR Aristolochiaceae, LA Lauraceae, PI Piperaceae, HE Hernandiaceae [13]

omies at the rank of family is constituted by the morphologically closely related Annonaceae and Myristicaceae [19, 20], the former containing benzylisoquinolines and the latter neolignans. This dichotomy continues upon passing to lower hierarchical rank. For instance Aristolochiaceae harbour the mostly American genus *Aristolochia* with benzylisoquinoline alkaloids and the Eurasian genera *Asarum* and *Saruma* with neolignans [13]. This is a common situation and shows the presence/absence criterium for chemical compounds or even for biosynthetic classes to be of limited value for classificatory purposes [1]. Indeed there are good reasons why morphology and chemistry in a particular plant lineage should not always be connected and we have observed chemical diversity for ecologically associated sympatric species of the same genus [23]

Another case of chemical dichotomy refers to polyacetylenes versus sesquiterpene lactones in the tribe Heliantheae of the Asteraceae The ancestral complex of the subtribes with chromosome numbers 8, 9 [24] seems more closely allied to modern series of subtribes with chromosome numbers 8, 9, 12, 16 with polyacetylenes than to other series with chromosome numbers 10, 11, 14, 15, 16, 18 with predominantly sesquiterpene lactones [Emerenciano, V de P, Ferreira, Z S and Gottlieb, O R., unpublished results]

As the foregoing results suggest, the clue derived from lignin and flavonoid composition and distribution is helpful in the rationalization of micromolecular evolution At least, in plant groups of high hierarchic rank, morphological evolution is accompanied by progressive oxidation of secondary metabolites belonging to a particular biosynthetic class As will be considered below, at infrafamiliar and lower rank, reductive gradients may oppose the general oxidative tendencies, possibly as one of several protective devices against oxidative degradation.

Micromolecular evolution among biosynthetic classes

The most conspicuous evolutionary trend in the gross morphology of land plants concerns the successive appearance of small weeds, larger herbs, shrubs and, finally, trees. This trend had attained or even had passed its climax with the primitive angiosperms and within this division the evolutionary polarity became inverted, woody plants being gradually replaced by herbaceous plants. These successional phenomena are paralleled by micromolecular compositions The ubiquitous flavonoids excepted, polyketides and terpenoids dominate the chemical compositions of bryophytes and pteridophytes Shikimate derived aromatics became numerically significant only in gymnosperms and attain predominance over other biosynthetic classes in primitive angiosperms Concomitantly here secondary metabolism reflects the trend from woody to herbaceous forms by inactivation of cinnamoyl/cinnamyl-derivatives through two phenomena (Fig. 12) [1]. (i) *extension* of the shikimate pathway by reduction of cinnamyl alcohols to allylphenols and propenylphenols and (ii) gradual curtailment of the final steps of the shikimate pathway The former alternative is most frequent in the primitive magnolialean block [25, 26] where oxidative oligomerization of the precursors leads to neolignans The first consequence of the latter alternative, the accumulation of phenylalanine and tyrosine, again very frequent in the magnolialean block, occurs also in the rosiflorean block [25, 26] Oxidative elaboration of these amino acids leads to benzylisoquinolines. Further shortening of the shikimate pathway is restricted to the rosiflorean block It leads to the accumulation of chorismic acid, the precursor of anthranilate- and of tryptophane-derived alkaloids, and of shikimic acid, the precursor of gallic acid- and ellagic acid-derived tannins

With gallic acid, the possibilities of diversifying the production of micromolecules through gradual curtailment of the shikimate pathway seem to be exhausted In

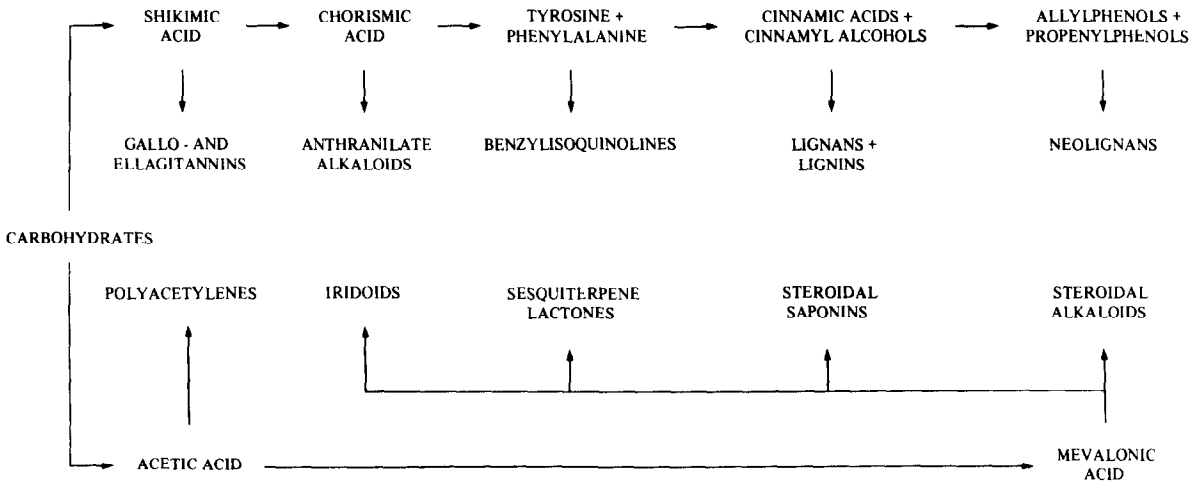

Fig 12

the most highly advanced, mostly sympetalous, angiosperms, shikimate-derived secondary metabolites play a relatively minor role. In these lineages, the full potential of acetate utilization leads to polyacetylenes, while mevalonate utilization leads to steroidal alkaloids, iridoid alkaloids, sesquiterpene lactones etc. In comparison with the polyketides and terpenoids of less advanced plant groups mentioned above, these compounds all show a high state of oxidation [cf. the sector on evolution within biosynthetic classes].

The statement concerning evolutionary curtailment of the shikimate pathway refers to its metabolites as precursors of micromolecules. Aromatic amino acids continue, of course, to be produced. Indeed according to Grisebach [27], 'it is still an open question whether there exists only one or several pools of α-phenylalanine supplying the precursors for protein synthesis, lignification, flavanoid formation, and the synthesis of other phenylpropanoids or compounds derived from them.'

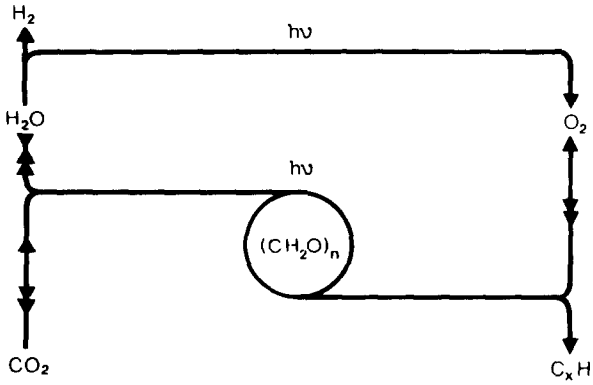

Fig 13 Principal causes for enrichment of the atmosphere in oxygen. Top photolysis of water vapour Bottom basic molecular phenomena of life (photosynthesis —▶—, respiration —▶▶—) and death (oxidative decomposition —▶▶— and anaerobic fermentation to C_xH_y)

DISCUSSION

The results suggest the possible dependence of micromolecular evolution on the variable oxygen content of the atmosphere or on the ecological pressure for diversification, to be discussed below.

Micromolecular structure and oxygen content of the atmosphere

The transformation of inorganic carbon (CO_2) into starting material for biosynthesis, i.e. sugar $(CH_2O)_n$, involves enzymic catalysts in the living cell and light as a source of energy. Oxygen liberated in this process promotes its reversal by respiration or oxidative decomposition of the organic material. If these complementary processes were indeed the only ones, no net increment in atmospheric oxygen would result. However, a small part (*ca* 0.1%) of the carbon incorporated into living organisms is, after their death, buried in the Earth's crust and transformed by anerobic bacterial fermentation into hydrocarbons. For each carbon atom thus removed from the cycle one molecule of oxygen is liberated (Fig. 13). Hence, since the introduction of photosynthesis early in biotic evolution, the net chemical result of life was the transformation of CO_2, produced by degasification of the Earth's interior, into oxygen. Photolysis of water vapour in the upper atmosphere and escape of the light hydrogen from the Earth's gravitational field may have been an additional source of oxygen in the past. The influence of this process on the composition of the contemporary atmosphere is thought to be negligible [28].

Initally, after formation of the Earth, oxygen freed from any source, was rapidly used up by ferrous and sulphide ions in the oceans and deposited in the form of iron oxides and sulphate minerals [29]. Only about 2 billion years (2 aeons) ago, the oxygen content of the atmosphere began to increase at first slowly and then, after the conquest of the continents by living organisms, about 0.5 aeons ago, at a faster rate. The present content of 21% supposedly was attained about 350 million years ago [30].

On the evolutionary time scale increasing diversity of forms, as indicated for instance by the increasing number of orders, correlates well with increasing oxygen content of the atmosphere up to 500 million years ago (Table 3)

Table 3 Correlation of the geological time in million years before present with the estimated level of atmospheric oxygen in percent of present level [30] and the biological diversity in cumulative number of fossil orders [31]

Time	Oxygen	Orders
400	100	
500		82
550	10	82
580		53
600		23
650		18
670	7	
700		17
800		15
950		14
1000		13
1300		12
1400	>1	9
1500		8
2000	1	6
2200		3
2800	0 1	
2300		2
3400		1
>3500	$<10^{-2}$	
4000	10^{-12}	

[31] Details concerning the variation of oxygen mass within the past 0.5 aeons have recently been disclosed [32]. Superposition on the respective graph of the geological times of the outburst of species diversity of plants and of animals (Fig. 14) reveals a surprising regularity. While the diversification of plant divisions coincides with oxygen maxima, this does not occur for animals The data do not refer to the time of emergence of a group, but to the start of its expansion in number of taxa For instance, with respect to angiosperms, the maximum oxygen content did not coincide with their time of emergence (yet disputed [35] but possibly coinciding with a period of relatively low oxygen concentration), nor with their maximal diversity (at present), but with the start of their expansion in number of taxa (120 million years ago). With respect to mammals, high atmospheric oxygen content coincided with their emergence (*ca* 160 million years ago [36]) and with their maximal diversity (at present), but not with the beginning of their expansion in number of taxa (60 million years ago). It seems relevant that at the outset of each of the three more recent periods of animal diversification, at pre-minimum oxygen masses, insects, modern insects and birds were favoured Interestingly insects are also rich in compounds which resemble plant metabolites rather closely. Besides, flying requires more energy, the result of consumption of organic matter by oxygen, than crawling

The times of minimum concentration of atmospheric oxygen coincide with the universal megaextinctions of species at the Cretaceous–Tertiary boundary (KTB) and the Permo–Triassic boundary (PTB) The causes of these extinctions are still controvertial One of the possibilities involves intraterrestrial events such as 'catastrophic vulcanism' [37], which would have acted directly on the atmosphere. Alternatively or concomitantly the impacts of large extraterrestrial bodies are postulated to have produced dust clouds that circled the globe causing blockage of sunlight and hence restricting photosynthesis [38]. Whatever the reason, the resulting obliteration of plant life should have been the disturbance favouring diversification of animals In contrast, the fairly rapid accretion of oxygen in the atmosphere, may have constituted the stress factor favouring the diversification of plants.

Let us now once more try to correlate morphology and chemistry. All fundamental biosynthetic pathways were already operative in primitive bacteria [39]. From the standpoint of secondary metabolism, the three most important comprise the acetate, the mevalonate, and the shikimate routes In organisms of the first, anoxic, stage (4–2 aeons ago), linear condensation of acetate and powerful reductions of the intermediates led to saturated and mono-unsaturated fatty acids, double bond formation in the latter involved dehydration, not dehydrogenation The formation of polyunsaturated fatty acids (requiring oxidation) and polyketides (dispensing with reductions and often also requiring oxidation) occurs additionally only in lineages of organisms originating in the second (2 to 0 5 aeons) and third (0.5 aeons to present) stages in an oxygenated atmosphere Again, condensation of acetate to mevalonate and reduction of further intermediates led to squalene, carotenoids and hopanoids, formation of the latter involving acid-catalysed cyclization, in organisms of the first stage The formation of oxygenated carotenoids and of steroids, the latter involving epoxide-mediated cyclization, occurs additionally only in organisms of the second and third stages, while the formation of even more highly oxygenated monoterpenoids (e.g. iridoids) and sesquiterpenoids (e g sesquiterpene lactones) occurs additionally only in organisms of the third stage Finally, condensation and rearrangement reactions led via shikimate to phenylalanine and tyrosine in organisms of the first and second stages Although considerations concerning involvement of oxygen here can also be made, it is especially relevant to plant life that post-phenylalanine and post-tyrosine metabolites (e.g. lignoids, flavonoids, benzylisoquinoline and indole alkaloids) occur additionally in organisms of the third stage.

This brief résumé intends to demonstrate that, in spite of all morphological disjunction between *Clostridium*, *Chlorella* and *Aster*, a few general condensation pathways are progressively developed in a continuum of further reactions, most of which involve gradual oxidation, and there is simply no reason why plant life should not operate within this general framework

The supposition can be substantiated by superposition of the curve of atmospheric oxygen evolution for the last 0 5 aeons [32] on data of cumulative land plant diversity as gauged by the analysis of fossils [33] Clearly major land plant diversifications happened in three successive stages (involving four plant groups) to coincide with the three successive oxygen maxima 430, 350 and 120 million years ago (Fig. 15).

The four crucial chemical stages which accompanied the genesis of the four plant groups concern the introduction of (i) flavonoids as shields against excess short wave

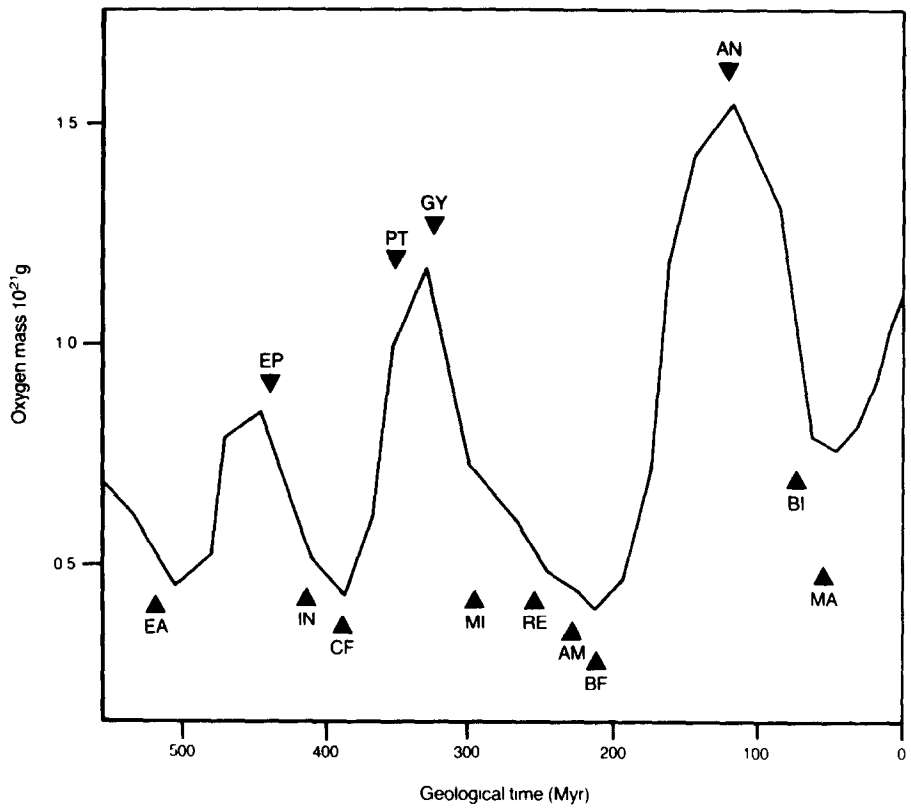

Fig. 14 Variation of atmospheric oxygen mass in the phanerozoic [32] and geological time of outburst of species diversity of plants: EP . early vascular plants, PT . pteridophytes, GY . . gymnosperms, AN angiosperms [16, 17, 33], and of animals· IN . . . insects, MI . modern insects [34], CF . . cartilagenous fish, BF . . bony fish, AM . amphibians, RE . . reptiles, MA . . . mammals [16], EA early marine animals, BI . birds KTB . Cretaceous–Tertiary boundary (67 Myr), PTB . . Permo–Triassic boundary (236 Myr).

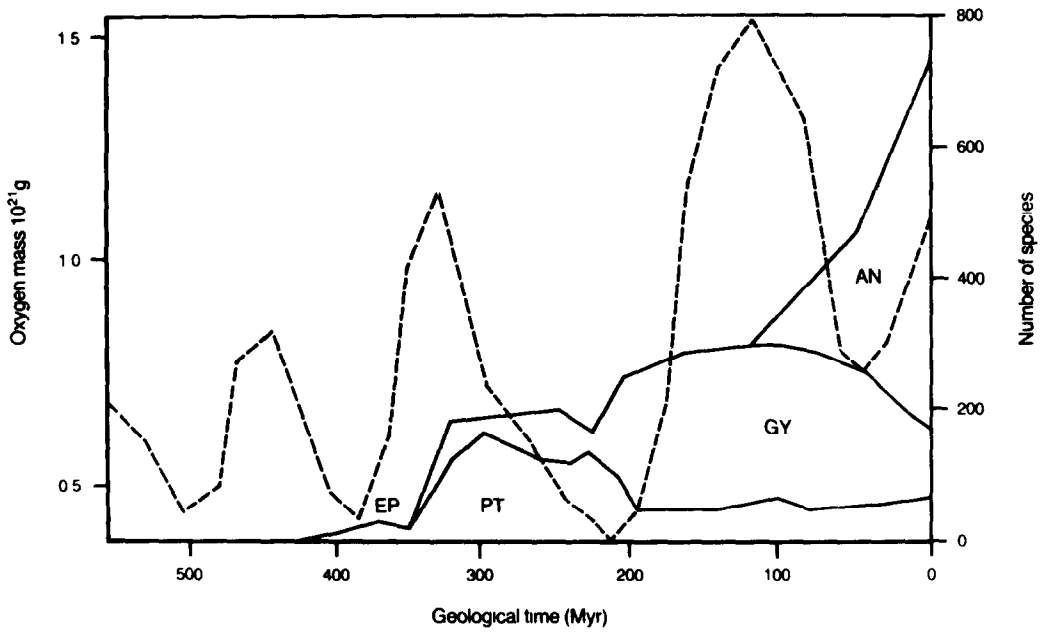

Fig. 15 Broken line. Variation of atmospheric oxygen mass in the phanerozoic [32] Solid lines· Vascular land plant diversity (in number of species) of the four groups of vascular plants (EP . . early vascular plants, PT . pteridophytes, GY . gymnosperms, AN . angiosperms) which have successively dominated the terrestrial flora, based on the compilation of *ca* 18 000 fossil plant species citations [33]

radiation from the sun and cutins as water-impervious coatings against desiccation which permitted the invasion of sunny, dry land surfaces by primitive tracheophytes; (ii) lignins to strengthen fibres for vertical growth and to construct vessels for the transport of water under pressure in pteridophytes, (iii) condensed tannins as general defense against pathogens and herbivores in gymnosperms; and, as discussed in the present paper, (iv) protection devices for phenols and other oxidizable compounds in angiosperms. The biosynthesis of flavonoids, cutins and especially of lignins needs oxygen, which, together with the other reported results, suggests the dependence of land plant evolution on the chemical composition of the atmosphere

The rationalization of this suggestion is far less simple than it may appear. Even 1% of oxygen in the atmosphere or even the amplitude of oxygen variation due to the diurnal photosynthetic rythm of a plant cell are each more than enough to justify the biosynthesis of the most highly oxygenated plant products Furthermore, oxygen diffuses passively through cell membranes, its solubility in the cytoplasm and hence its concentration being of course identical in all plants Thus if a correlation of oxygen content of the atmosphere and the oxidation state of plant metabolites exists at all, this must be due to efficiency of oxygen transfer, and hence requires the evolution of appropriate enzymatic systems In order to understand the meaning of this sentence we have to renew our concept concerning the turnover of secondary metabolites.

Micromolecular structure and co-evolution with the environment

Secondary metabolites are mostly oxidation products of primary ones, i e the biosynthetic sequences usually start with condensations and reductions (to primary metabolites) and are finalized by oxidative steps. In this process oxygen not only operates directly, by oxidation of an appropriate precursor, but also indirectly, by establishing conditions for skeletal transformation. As with so many natural processes, this is also a cyclic one, because if allowed to proceed, these oxidative steps lead on to the recovery of starting materials, e g acetic acid and carbon dioxide. Thus oxidative pathways to micromolecules may occur with comparable potency in all plants. However, unless the more highly electron-rich compounds formed are protected, e g. by etherification (Fig 16), Schiff base formation (Fig. 17) or partial hydrogenation (Fig 18), a considerable number of degradation reactions leading eventually to CO_2 will follow [40]

A demonstration of the protective role against oxidative degradation played by methylation is provided by the flavonoid distribution in *Derris* and *Lonchocarpus*, two closely related genera of the Fabaceae Their forest

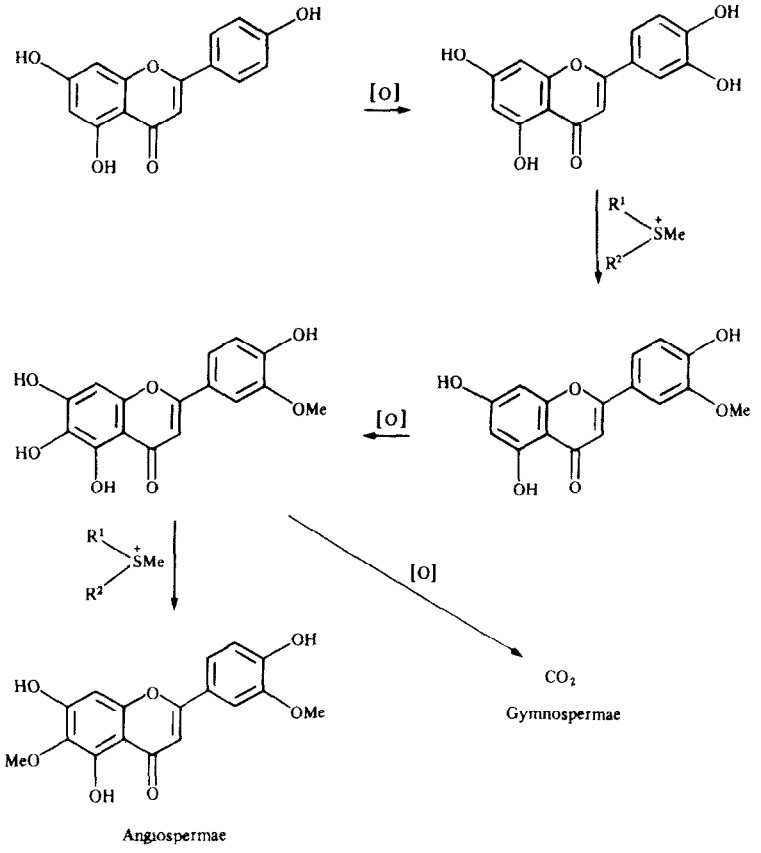

Fig 16

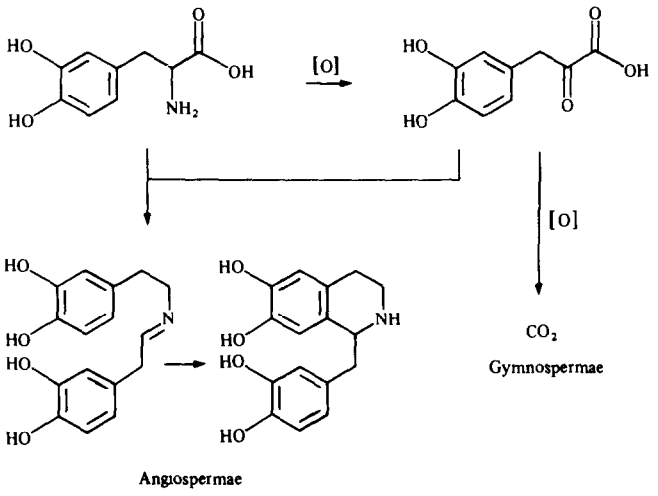

Fig. 17.

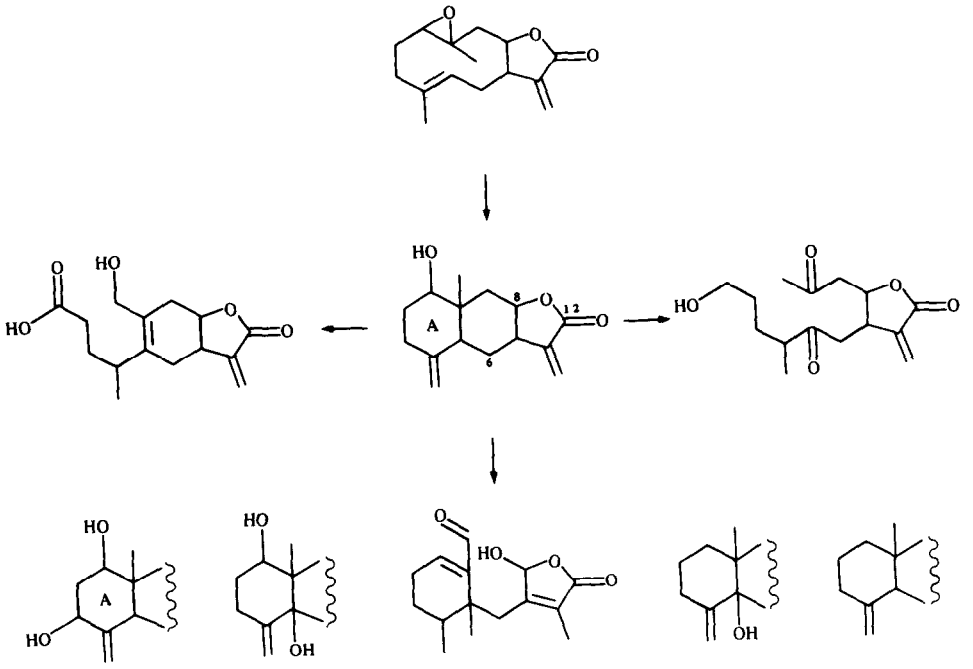

Fig. 18.

species, endowed with clustered flowers, contain rotenoids of similar oxidation (O) and O-methylation (M) levels. In view of the predominantly south-east Asian location of *Derris* and the predominantly south American location of *Lonchocarpus* this indicates chemical stasis over an enormous geographical range and suggests high O and M values to be primitive characteristics in the plant group. The radiation of *Lonchocarpus* from forest to savanna is accompanied by morphological reduction to paired flowers. Concomitantly the more highly oxidized and methylated flavonoids of forest species are replaced by more highly reduced and hydroxylated flavonoids [41], methylation and reduction being alternative protection devices. It is noteworthy that evolutionary polarity here, at low hierarchical rank, progresses by reduction of micromolecules.

This is by no means a special case and was observed equally for the distribution of sesquiterpene lactones in tribes of Asteraceae [7, 10]. Here pertinent EA_s and EA_o parameters are represented along two gradients, one encompassing the tribes of the subfamilies Lactuceae and Asteroideae—group 1 and the other encompassing the tribes of the subfamily Asteroideae—group 2 (*sensu* Wagenitz [42]). For both gradients, in the direction from high to low EA_s and EA_o sesquiterpene lactone derived values, decrease of *O*-glycosylflavonoids *versus* increase in *O*-methylflavonoids is observed. If this trend in replacement of *O*-glycosylation by *O*-methylation is taken

to indicate evolutionary advance, as has been advocated [43], this is again accompanied at subfamiliar rank by gradual reduction of micromolecules, i e. stabilization of sesquiterpene lactones.

Structural inspection of micromolecular constituents in ferns and conifers reveals that very few stabilizing enzymes are present in the corresponding divisions In gymnosperms the *O*-methyltransferase is highly specific for the generation solely of a guaiacyl unit [44]. In contrast, the protective systems of angiosperms are effective in the deactivation of many specific reaction centres A caveat is in place concerning the term deactivation This is meant to describe only the lessening of turnover rates in the cyclic process. Even *O*- and *N*-methyl compounds for example are subject to oxidative demethylation [45], a fact which further increases the diversity of the biosynthetic reaction products in angiosperms The existence of partially stabilized intermediates explains the 'exaggerated' as opposed to parsimonious versatility of angiosperms leading to a far greater collection of products than seems reasonable to expect for interaction with particular pathogens and herbivores.

Two examples will clarify this concept The biosynthesis of neolignans (Fig 19) involves propenylphenols and allylphenols protected by *O*-methylation at strategic positions, clearly an evolutionarily determined phenomenon Oxidation can then lead only to a few of all possible radicals, the again enzymatically oriented coupling of which leads to a hypothetical intermediate. From here on seemingly any mechanistically plausible reaction may occur and three totally different structural types of neolignans are formed [46]. Analogously for the biosynthetic derivation of oxidative oligomers in the genera of the Gnetatae (Fig. 20) [47] systematic coherence is given for the entire group by identical starting materials and for each genus by identically disposed micromolecular substructures Various skeletal complements, attached to these substructures, determine the diversity of the final products

CONCLUSION

The lignification process and the biosynthesis of micromolecular classes in terrestrial plants are connected. This is evidenced by parallel distribution trends of macromolecular lignins and of micromolecular lignoids and by the evolutionary increase in the oxidation level of lignins and of micromolecules belonging to given biosynthetic classes. The structures of lignins and of micromolecules suggest the influence of the oxygen content of the atmosphere in their biosynthesis at the time of emergence of the respective plant group Nevertheless, oxygen does not seem to act directly in micromolecular evolution. The driving force behind its action may stem from the facts that the higher the oxidation level of a secondary metabolite (i) the smaller the requirement of energy for its

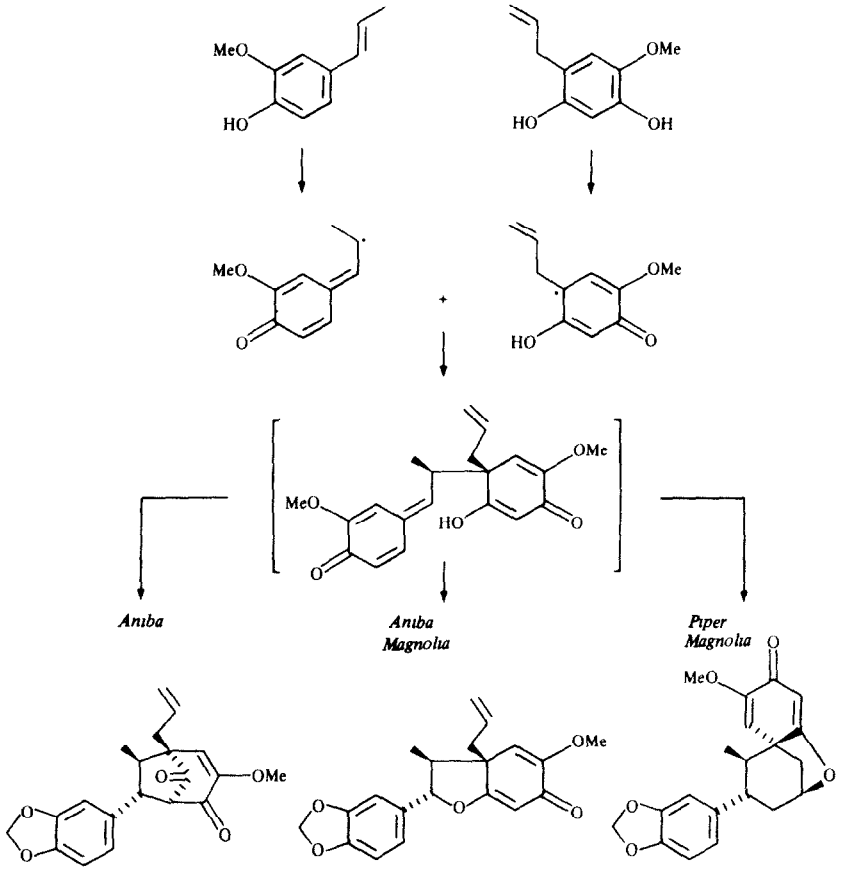

Fig 19

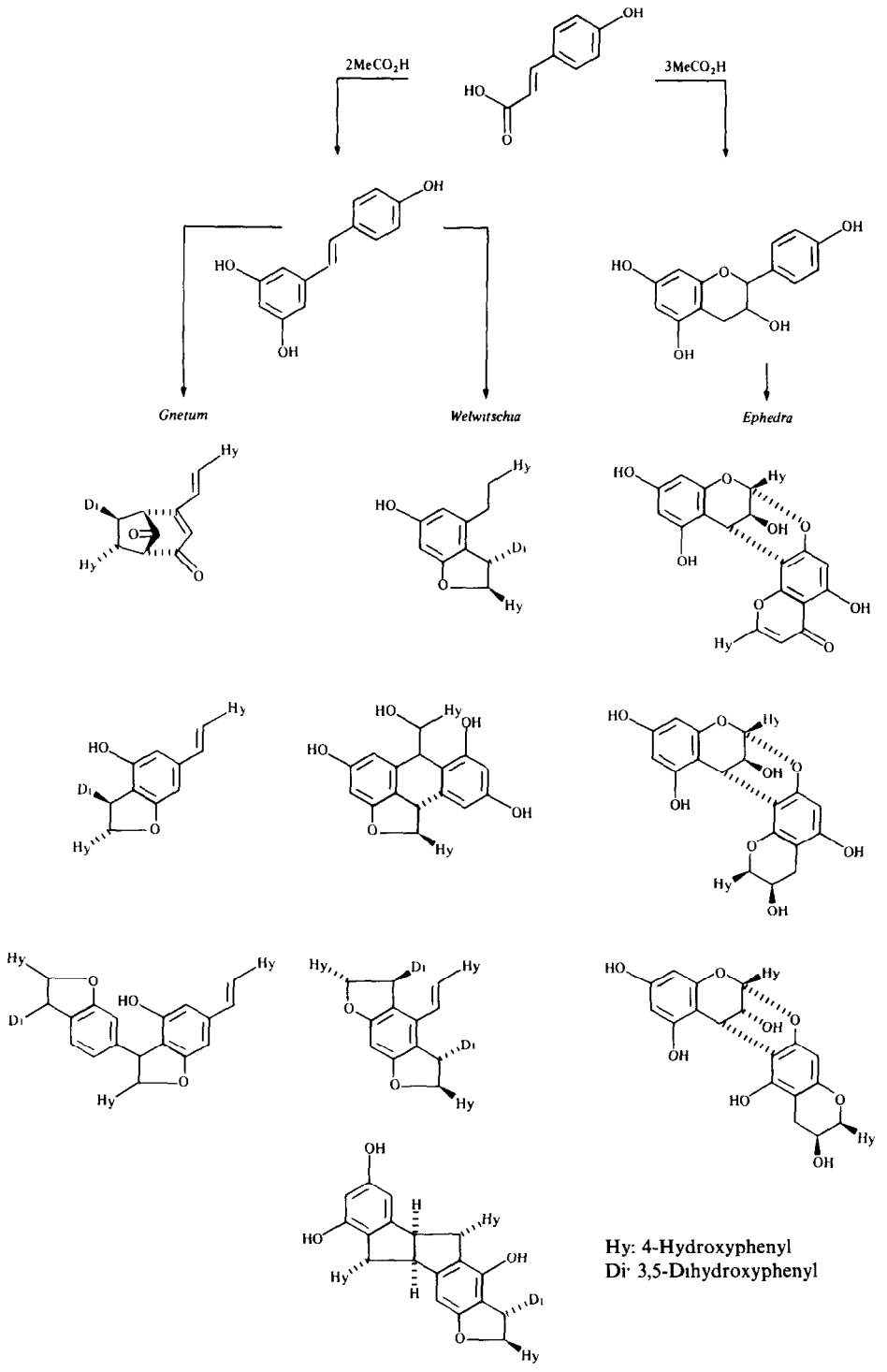

Fig 20

conversion back to starting material (better performance as antioxidant [48]), (ii) the greater the economy involved in such recovery of starting material during senescence of a plant's organ [49], and (iii) the greater the ease of its diversification by chemical transformation and rearrangement. Besides, oxygen should trigger the evolution of enzymatic protection devices, performing selective etherification, Schiff base formation or reduction, for (i) the regulation of a micromolecule's half life during its oxidative turnover and (ii) the orientation of a micromolecule's biosynthetic pathways. Both phenomena are essential in the diversification of secondary metabolites and hence requisites for the flexibility of an organism's adaptation to the environment

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