The general rôle of terpenes and their global significance

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<u>Abstract</u> - Terpenes are universally present in small
amounts in living organisms, where they play general rôles in membranes.
On the other hand, they are also accumulated in many cases, and it is
shown that the extraordinary variety they then display can be due to
ecological factors playing an evolutionary rôle. Examples are given to
show that such an ecological rôle can be either "obvious", when these
secondary metabolites are biologically inactive, or "hidden", when they
are toxic and potentially deterrent, or simply active as hormones.

INTRODUCTION

Terpenoids are a ravishment and a nightmare. A nightmare for the student trying to memorize
hardly different structures and crazy trivial names, and a ravishment when he discovers that
a few basic rules are enough to "understand" thousands of structures and to exclude from
serious consideration many, that would otherwise apparently fit the available structural
data. A ravishment also, when we see the white of betulin in the bark of birches, the
orange, yellow, crimson or red of carotenoids in a basket of peppers or in a flight of
flamingoes; a ravishment when used in the proper combination in a spice or a perfume, to lure
a beetle (or ourselves) to a prospective mate, but a nightmare, when one is confronted with
the nagging question: **"Why such a diversity ? Would Nature not have worked, had it been
simpler ?"**.

I shall not answer this question directly, of course. But I shall use the experience accu-
mulated in our group to present a plausible global picture of variability and homogeneity
within the terpenoids. The major results on which I base these views are:

- the discovery, in sediments, of many types of molecular fossils of steroids and terpenes,
described at the previous IUPAC Symposium in New Delhi (ref.1),

- the discovery of the ubiquity of geohopanoids, of their extraordinary abundance, of the
widespread occurrence of biohopanoids in Procaryotes, and the hypothesis of their rôle of
procaryotic surrogates of cholesterol, described at the IUPAC Symposium in Varna in 1979
(ref. 2); this remains an extraordinary case of discovery of a family of biologically impor-
tant substances <u>after</u> and because their molecular fossils had been isolated from sediments
and found to be varied and abundant;

- the confirmation of the stabilizing effect of biohopanoids, and of many other types of
polyterpenoids (carotenoids, tricyclopolyprenols, archaebacterial lipids), on phospholipidic
membranes, and the hypothesis of a phylogenetic derivation of polyterpenes, presented at the
IUPAC Symposium in Kyoto in 1988 (ref. 3),

- and the discovery and the study, by our group and mostly others, of many different types of
biologically active terpenoids, most of them characterized by extensive oxygenation patterns
(ref. 4).

I shall first summarize very briefly the conclusions we had already arrived at, to "justify"
the **ubiquity** of terpenes in living organisms, and then propose an interpretation of their
diversity. I shall not attempt to justify my statements with other references, and shall ask
the reader to accept the apparent resulting dogmatism. In several of the other lectures and
posters at this Symposium, there will be plenty of illustrations of my thesis, which is
proposed more as a "lecture guide" than as an original contribution. This is also why, for
the first time, I present a purely verbal contribution, without any formula.

THE UBIQUITOUS PRESENCE OF TERPENES AND THEIR GENERAL ROLE

The fact is that terpenes appear to be present in all living organisms. We have come to the conclusion (ref. 2, 3) that this permanency of presence comes from the fact that they exhibit, in all living organisms, a constant function: to impart suitable mechanical characteristics to cell membranes. This is achieved in higher Eucaryotes by the insertion of the rigid molecules of **cholesterol** in the fluid phospholipid membrane matrix. In Protozoa, the same rôle can be achieved also by the biogenetic precursor of cholesterol, cycloartenol, or by a pentacyclic triterpene, tetrahymanol. In Procaryotes, cholesterol is normally absent, and membranes contain **polyterpenic surrogates**: either hopane or bacteriohopane derivatives, or α,Ω-dipolar carotenoids, or maybe tricyclopolyprenols and isoarborinol (revealed by their molecular fossils). In every case, efficacy as a membrane component requires adaptation of molecular features: these molecules are all partially rigid, amphiphilic, and of dimensions permitting cooperative van der Waals interactions with the phospholipidic chains.
The case of Archaebacteria is in fact the most revealing, in that their phospholipids are constituted exclusively of polyterpenic ethers, generally partially or completely saturated, and often Ω,Ω-linked into symmetrical C_{40} chains, spanning the membrane perpendicularly, or U-shaped.
Other polyterpenes are involved in quite general functions linked with the universal presence of membranes: farnesol, involved in alkylation of proteins as a step in their maturation, the ubiquinones, involved in electron transfer, dolichols, very long prenols (up to ca. 20 prenyl units), essential partners in the glycosylation of proteins; all these complex functions are probably more recent than the primary function of membrane formation or stabilization.

In summary, terpenoids are universally present in organisms and appear to play general functions in their membranes. One can derive a hypothetical scheme assigning them a putative rôle in prebiotic membrane fromation, and we have previously presented the hypothesis of a progressive evolution through discrete steps, with the successive enrolment of novel enzymatic reactions (ref. 3). Further refinement of this theory will have to await the results of structural studies of several of the enzymes involved, now underway in several laboratories.

THE DIVERSITY OF TERPENOIDS AND THEIR HOMOGENEITY

The diversity of Natural Products stems from two very different and general processes. The diversity of combinations of very few basic elements ensures the diversity of the essential biopolymers, proteins, nucleic acids, polysaccharides : the basic elements are the 20-odd protein aminoacids, the 4 nucleosides, a handful of monosaccharides. The biopolymers are words, indefinitely diverse but composed of a small number of letters, written in three different alphabets. For "secondary metabolites" however, the diversity is intrinsic: hundreds of flavonoids, thousands of alkaloids, tens of thousands of terpenoids, each one with its own skeleton and functional arrangement. They are as diverse as hand-made objects or art works, but not haphazardly so: their structures betray their origin and, more importantly, their diversity hides, in each of their families, a basic homogeneity: the analogy or even identity of their precursors and of the mechanisms of their biosynthesis. We shall first summarize classical results "explaining" the homogeneity of terpenoids, and then show that, from the "first level" polyterpenoids, two mechanisms appear to be at play to introduce heterogeneity: a random structural drift, in the case of products of excretion (resins, latices, etc), and the fixation of biologically active products of catabolism into physiologically or ecologically essential metabolites.

The Biogenetic Isoprene Rule of the Zürich School has given us an universal interpretation of the structures of all known terpenoids: they derive from the simple acyclic polyprenols by "normal" chemistry, mediated by enzymes but involving "normal" reaction mechanisms. Of these mechanisms, the most important ones for the formation of the skeleton are the addition of a carbenium ion on a double bond, and the 1,2-migration on a carbenium ion: these ensure not only the formation of the simpler acyclic precursors, the polyprenols, but also their cyclizations and the rearrangements of cyclized products.

$$C^+ + C=C \longrightarrow C\text{-}C\text{-}C^+ \quad ; \quad C\text{-}C\text{-}C^+ \longrightarrow C\text{-}C^+\text{-}C$$
$$\phantom{C\text{-}C\text{-}C^+ \longrightarrow}\ \ \ C \phantom{\longrightarrow C\text{-}C^+} C$$

We have shown how a coherent phylogenetic scheme can be built for all major groups of terpenoids by the successive enrolment of new enzymatic systems to allow duplication head-to-head (to the archaebacterial lipids), or tail-to-tail (to squalene or prephytoene), and by the progressive evolution of the "same" cyclases to yield, from the same or similar substrates, quite different end-products (ref. 3).
The Biogenetic Isoprene Rule has in a way taken the fun out of the structural investigation of terpenoid skeletons: all the novel ones, found after the Rule had been formulated, are consistent with its pre-requirements, and would have been "predictable". However, the initial skeletons, once formed, can be further modified, essentially by oxidations, responsible for functional variations and for degradations.

VARIABILITY AMONG BIOLOGICALLY "INACTIVE" PRODUCTS

A first level of variability is achieved among terpenoids segregated from the cells producing them, by excretion into resin canals, laticifers, secretory glands, barks. In these cases, the exact nature of the constituents appears to be irrelevant, but their presence is constant, and therefore probably significant. In a given species, the spectrum of constituents is relatively constant, so that this spectrum can be used as a chemosystematic criterion.

For instance, Conifers contain resin canals filled with a mixture of monoterpenes (pinenes, carenes, etc), minor amounts of sesquiterpenes (longifolene, caryophyllene, etc), and diterpenes (resin acids, kaurene, etc). In Labiatae again, similar specific mixtures of mono and diterpenes are found. In those other major producers of resins, the Dipterocarpaceae, monoterpenes are absent; their oleoresins contain sesquiterpenes and triterpenes, whereas in Caesalpiniae, they contain mostly diterpenes, often of the configuration opposite to that of the resin acids, which is also the case for the sesquiterpenes present in many liverworts. In tree barks, triterpenes are often present, and are then very diversified, but mostly with a 3-keto group. In all these cases, the terpenoids involved appear to be very inert, and not to undergo any metabolic transformation: they are end-products. They are also usually devoid of any marked biological activity, and their very diversity makes it of course impossible to assume that each of them should have, in the plant producing it, a decisive physiological rôle. The most probable interpretation of the interplay of constancy (taxonomic groups containing products of the same general type) and of variability (related taxa containing variable substances) is that the gross nature of the system is important, but not its precise molecular composition, and that its importance is due to ecological reasons: barks are made more hydrophobic by the triterpene ketones they contain; resins, hardening when exposed to air by injuries to the plant,
seal cuts; terpene-filled hairs or canals provide protection against predators (though of course not against specialized ones, which have become adapted to live on Conifers or Labiatae, etc.), but the nature of the terpenes filling them can freely drift. At the molecular level, the variations observed are probably excellent examples of neutral evolution, while the constancy of the general features observed can explain their fixation (if it is really the effects we are able to recognize which are decisively efficient as competitive characters).

VARIABILITY AMONG BIOLOGICALLY ACTIVE TERPENOIDS

An apparently more obvious degree of ecological protection (but one not better demonstrated) is provided to many organisms by toxic metabolites. Some 30 years ago, it was considered a rule that toxicity was associated with nitrogen-containing functions, as in the alkaloids. Cases like those of the toxic, but nitrogen-free, cantharidin, phorbol or andromedotoxin were considered as rather extraordinary. In fact, oxygenated derivatives of precursor terpenes, obtained by the normal catabolic routes, are very often biologically active in many different ways, which leads to very different conclusions (ref. 4).
A very frequently observed biological activity oxygenated derivatives of terpenes display is to regulate, by a feed-back mechanism, their own biosynthesis; thus, many of the hydroxy-sterols produced from sterols by the action of cytochrome P-450 oxidases are feed-back inhibitors of the HMGCoA-MVA step or of other steps in the biosynthesis of cholesterol. A spectacular case is that of the fungal triterpenes, in which lanosterol, the precursor of their sterols, can be accumulated and hydroxylated in practically any position, giving products of moderate or high cytotoxicity associated in particular with inhibition of sterol biosynthesis.
This implies that there are receptors for these oxygenated derivatives, and it appears that this makes possible the elicitation of remarkably varied biological activities. One can mention for instance:

- the sesquiterpene toxins of fungi (mycotoxins: neuro-, entomo-, phyto-, genotoxins...),
- the irritant and cocarcinogenic oxygenated diterpenes of the latex of Euphorbiales (phorbol, ingenol, etc),
- the cardiotoxic oxygenated and degraded steroid glycosides of Digitalis, or of Scilla, or of toads,
- the bitter, antifeedant, cytotoxic, degraded triterpenes of Meliaceae or Rutaceae,
- the hemolytic and foaming saponins of Liliaceae or Dioscoreaceae, or of sea cucumbers (lanosterol-based) or of sea stars (cholesterol-based)
- the bitter and cytotoxic triterpenes of Cucurbitaceae, the cytotoxic, allergenic, sesqui- or diterpenic lactones or quinones of Compositae, the toxic diterpenes of Ericaceae, the pungent sesquiterpenic aldehydes of Hepaticae or higher plants, etc, etc.

In all these cases, it looks quite probable that the presence of these substances leads to some level of protection of the organism producing and accumulating them, to some (syn)ecological advantage.
In fact, exactly the same mechanism: the specific accumulation of the products of an erratic degradation of "neutral" precursors, can also account for the nature of many of the terpene-derived hormones, such as our own sex or corticoid hormones, but also the juvenile and moulting hormones of insects, the mating hormones of Oomycetes, the gibberellins of

plants, etc. In a similar vein, cholesterol itself, an essential constituent of the membranes of Eucaryotes, is the product of the oxidative catabolism of its triterpenic precursors : lanosterol in fungi and animals, cycloartenol in plants and at least some Protozoa. In these cases, the remarkable outcome is that some metabolites of an universal component of membranes becomes also essential, but for some more elaborate, more specific function. This is an extreme case of "tinkering".

In short, it appears to be a general rule that many products of the normal oxidative catabolism of terpenes are biologically active, and that this can become a positive factor for the organisms producing them, either by a direct action (biochemical, biophysical or physiological effects), or by a positive effect in the struggle for life (ecological effects).

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