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The irones and their origin

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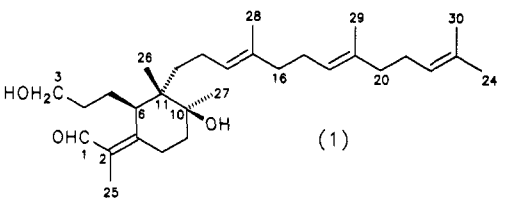
Abstract - The violet fragrance of stored dry rhizomes of certain Iris (sword lily) species is due to the irones, homologues of ionones. The whole family is derived from the parent compound iridal. From the various iridals isolated and characterized, a biogenetic sequence was proposed which is currently under scrutiny. From the knowledge gained it is now possible to obtain the valuable irones in higher yields from their natural sources.

Irones are the pleasantly smelling terpenoids of the orris oil, important in perfume industry, that is extracted from the rhizomes of certain sword-lily or *Iris* species (*I. pallida* var. *dalmatica*, *I. germanica* and *I. florentina*) in which they accumulate during storage. They have been identified as homologues to the ionones, cyclogeranyl acetonides easily accessible from abundant natural citral [1]. Ionones occur naturally *inter alia* in the scent of violets and are formed from carotenes by blooming algae. The distinguishing feature of the irones is an extra methyl group in position 5, as was shown simultaneously and independently by *Naves* [2] and by *Ruzicka* [3].
The extra methyl group of the irones is an intriguing biogenetic pecularity, worth studying. It was soon found by *in vivo* experiments with *Iris* plantlets that it is not derived from acetate as the rest of the terpene ring structure but from the methyl group of methionine - apparently by a one-carbon transfer reaction from adenosyl methionine [4].
This generates the question on the acceptor molecule, and in pursuing this search we found the precursors in a class of hitherto undetected triterpenoid constituents of the unsaponifiable fraction of lipid extracts of *Iris* rhizomes, which we named the iridals [5]. They proved to be uncommon structures overlooked till now although occurring in appreciable amounts. On vigorous oxidation they yield odoriferous irones or odourless dihydroirones which are also accessible by chemical reduction of the former.



(1)

Figure 1: Iridal

The ecological service of the iridals to the plant in nature is not known. All of them are very bitter tasting, amphiphilic compounds which may act either as deterrents, as antibiotics, or as membrane plastifiers. The characteristic feature common to all of the iridals is a multi-substituted cyclohexane ring with a long side chain at one corner (C-11 in our numbering, which follows the numbering of squalene, see *Figure 1*) and an acrolein group *meta* to it (C-7) as well as a hydroxypropyl chain *ortho* to it (C-6). The latter two substitutions are typical fragments of a *seco* A-ring of triterpenoids. If this ring were closed, a striking similarity would become apparent to ambreine from spermacetic ambergris which also possesses a comparable but terminally cyclized homofarnesyl substitution [7]. However, in the iridals the methyl group at C-6 has migrated into position 11.
The iridals occur in astongishingly large quantities in Bligh-Dyer extracts of shredded rhizomes of all the *Iris*-species studied. They make up to 1.5% of the wet weight but are present in many different variations and often esterified with fatty acids C_{12} to C_{20} at C-3; part of them - particularly those with conjugated side chain or secondary ring formations - turned out to be highly labile and required special precautions in work-up. The lipid extracts are fractionated by chromatography on silica gel and then on reversed phase columns by LPLC to yield the colour- and odourless iridal compounds - mostly in form of resinous solids. Up to now only one of the iridals has been crystallized, but this helped greatly in elucidating the structure and absolute stereochemistry of all the closely related natural products [6].

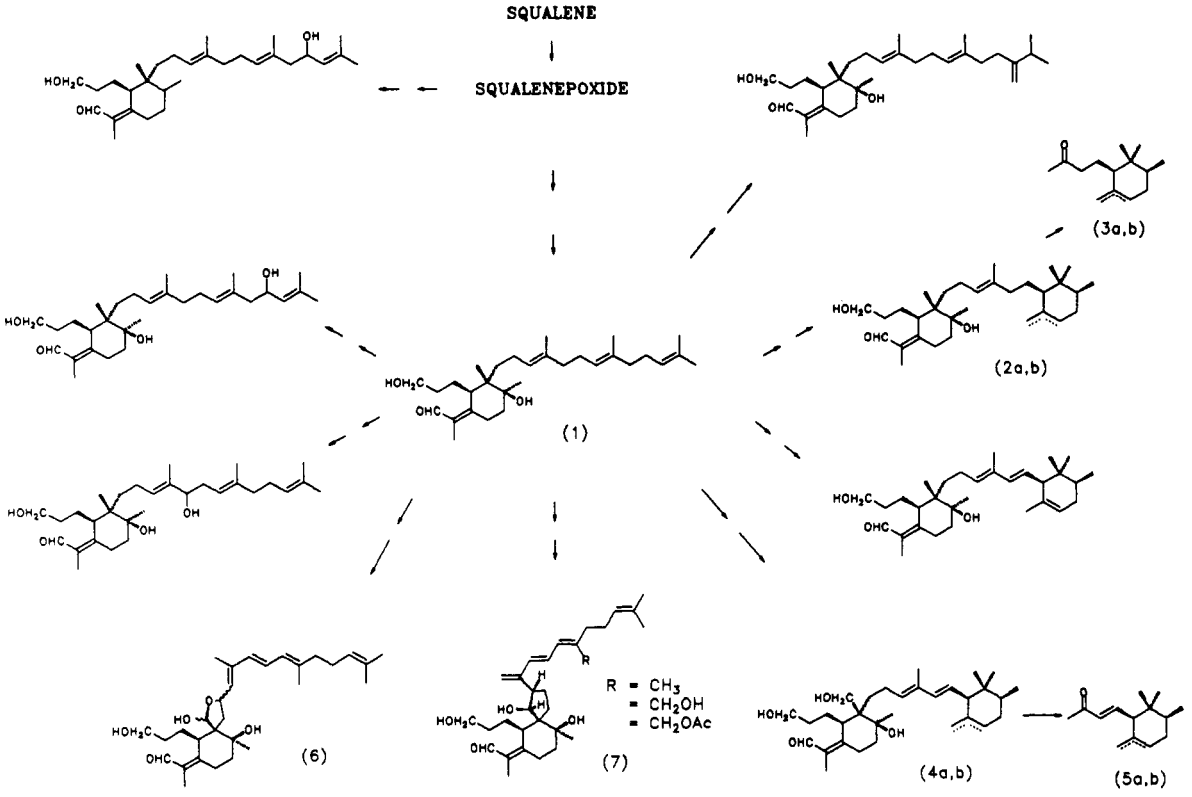


Figure 2: The iridals and their biogenetic connection

Detailed spectroscopic analysis, combined with chemical conversions and degradations proved the array of compounds separated to be unmethylated C-30, or methylated C-31 triterpenoids (*Figure 2*). All but one of the latter compounds bore the methyl group in an extra (E) ring structure preforming the characteristic irone ring; they were termed cycloiridals. The cycloiridals occur in different isomers, yielding α- and -irone on oxidation with relatively mild oxidants such as permanganate if the side chain is unsaturated. Thus, 26-hydroxy-22-methyl-α-cycloirid-16-enal (4a) which is the main component of *I. pallida* extracts gives rise to α-irone (5a), 26-hydroxy-22-methyl- -cycloirid-16-enal (4b) from *I. florentina* yields -irone (5b). Indigenous *I. germanica* almost exclusively contains the two isomeric more highly saturated 22-methyl-cycloiridals (2a,b) which, upon vigorous oxidation, *e. g.* with *Jones'* reagent, afford α- and -dihydroirone (3a,b). It is assumed that also the 26-hydroxymethyl group is auxiliary in labilizing the irone precursors.

The inventory of the iridals in *Iris* species, traced by the characteristic acrolein absorption and identified unequivocally by 2D-^{1}H- and ^{13}C-NMR is impressive, and *Figure 2* gives the examples found till now. As seen, there are homofarnesyl and methyl-homofarnesyl substituents with different degrees of unsaturation in form of separated double bonds and double and triple conjugations. Oxygen is introduced at different pronounced places such as at the methyl group in position C-11 (to form the system-labilizing 26-hydroxymethyl group) or at the electron-deficient positions α to the double bonds. Also, new cyclizations take place by refolding the side chain back to the ring to yield hemiacetals (6), derived from 26-formyl iridals by attack of an OH-group of the chain, or even to make a true spirane structure (7) with a cyclopentane ring by C-C-cycloaddition, requiring attack on that carbonyl group by an olefinic carbon. These are the first known spiro triterpenes in natural products.

Particularly rich sources of such triene- and odd iridals are *I. pseudacorus*, the swamp lily, occuring on boggy soil and *I. foetidissima*, the stink lily. Chromatography, combined with mass spectrometry and nuclear resonance spectrometry in one and two dimensions, finally by NOE measurements proved beyond doubt the novel spirane structure (7) and also the other chemical and geometrical details [8].

Besides these novel and unusual structures there are several intriguing points of interest in the chemistry and biogenesis of the iridals:
1. Closer examination of the irones obtained on oxidation from cycloiridals isolated from different varieties of the same species but growing at different geographical locations revealed them to be enantiomers yielding either dextro- or laevorotatory irones. There seem to be genetic or/and environmental factors which decide upon the stereochemical course of the enzymatic reactions leading to the irone precursors in these cultivars. As an example, all the Italian cultivars of *I. pallida dalmatica* contain the precursors of dextrorotatory irones, the indigenous *I. pallida* and the Moroccan *I. germanica* those of laevorotatory irones. The chirality of the five optical centres of these compounds (C-6,10,11,18,22) has been elucidated to be *RSSSR* and *RSSRS*, respectively [9].
2. The biogenetic scheme already displayed in *Figure 2* is supported by several lines of experimental evidence with radioactively labeled compounds and precursors. Thus, 2-(*^{14}C*)-acetate, 2-(*^{14}C*)-mevalonate and 4,8,12,13,17,21-(*^{3}H*)-squalene injected into young *Iris* plantlets, were incorporated into the triterpenoid fraction at acceptable rates proving the squalenoid origin of these natural products [4].
3. A mechanism for the introduction of the methyl group from activated methionine (Ado-Met) to the terminal double bond of an open chain precursor is shown in *Figure 3*. The radioactivity from (*^{14}C*):(*^{3}H*)-methyl-methionine

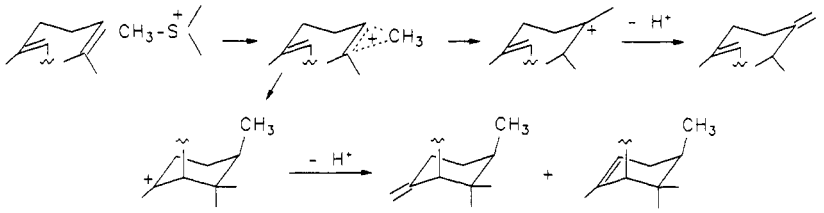


Figure 3: Mechanism of the methylation

occurs solely and with the relative specific activity unchanged in the methylated cycloiridals. The most plausible process for this addition of CH_3 without loss of tritium would be a mechanism in which an enzyme-bound intermediate cation is formed. Simultaneously, the carbenium ionic charge may be left either at C-22 or after cyclization at C-19. In the first case an open chain 22-methylene compound will be formed as the end product by stabilizing proton elimination. Such an iridal has indeed been found. The carbenium ion at C-19 will stabilize by proton abstraction from C-20 or C-29 to form the immediate α- and -irone precursors. The stereochemistry of the ring compound depends on the folding of the homofarnesyl side chain into the enzyme pocket. As shown in *Figure 4 cis*-irones are derived from the thermodynamically favoured chair form of the terpenoid side chain, whereas the boat form leads to the *trans* products. The synthesis of enantiomers may be explained

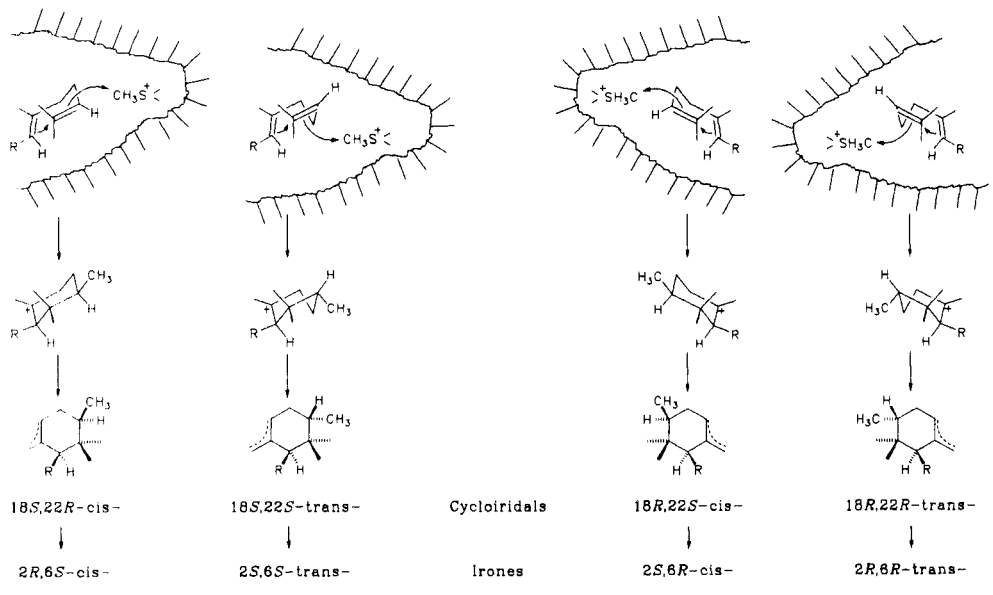


Figure 4: Mechanism of the irone ring formation

by different (sub-)sets of enzymes, the active sites of which are mirror images of one another. Thus, in *I. pallida dalmatica* the dextrorotatory 2*R*,6*S*-cis and 2*S*,6*S*-*trans* irones are formed and one prediction of this scheme is that the *trans*-irones accompanying the 2*S*,6*R*- *cis*-irones - if ever found - should have 2*R*,6*R*-geometry.

4. Still more complex is the mechanism of biogenesis of the highly substituted B-ring. A possible explanation is given in *Figure 5*. When looking at ambreine it is evident that both structures are closely related. However, whereas ambreine is derived by proton-triggered cyclization of squalene, 2,3-epoxysqualene undoubtedly is the precursor of the iridals as shown by appropriate labeling experiments. Certainly a bicyclic intermediate is formed this way, the A-ring of which is subsequently opened to the iridal skeleton. The intermediate formation of a bicyclic ketone can be excluded, since the radioactivity of 3-(^{3}H)-2,3-epoxysqualene injected into young *Iris*-plantlets is found in the iridals [10]. The possible iridal precursor (8), (^{14}C)-labeled in the methyl group at C-8 [11], and the natural product (9), isolated from gum mastic and tritiated at C-3 [12], are not incorporated. However, no definite statement can be made, whether such bicyclic diols are intermediates of the iridal biosynthesis, since the synthesis of (8) was not stereospecific and (9) has the "wrong" stereochemistry at C-8 with the OH-group in a position that would interfere with the rearrangement to the iridal moiety. Another plausible mechanism for the formation of the substituted B-ring is the direct sigmatropic rearrangement of a charged bicyclic intermediate obtained by proton-induced cyclization of 2,3-epoxysqualene (*Figure 5*). By this scheme not only the unusual substitution but also the stereochemistry of the compounds may be explained: C-1 and C-10 will have to be oxidized - the latter with inversion of its configuration -, and C-3 must be reduced to obtain the basic iridal.

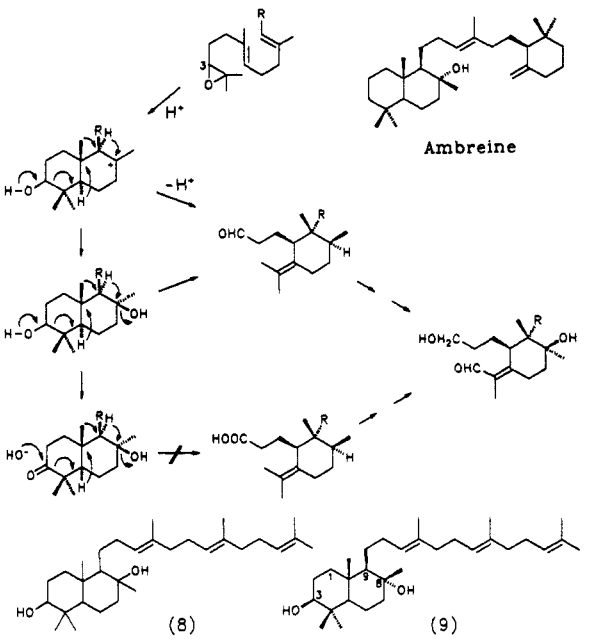


Figure 5: Possible pathways to the iridals

Further experiments to elucidate on this basis the biosynthesis of the iridal skeleton and to study the reactions of these unusual triterpenoids are under way. It is a challenge to clarify the mechanism of their formation and, of course, their meaning for the life of the plant.

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