# SOME STUDIES IN THE BIOSYNTHESIS OF TERPENES AND RELATED COMPOUNDS

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Cyclic terpenes as a class of natural products display a bewildering array of structural types. Well over a hundred different carbon frameworks have been detected so far, many new substances are being currently added to an already impressive list of compounds, and even several representatives of the long-sought group of sesterterpenes have at last made their appearance on the chemical scene[1–4]. In spite of this almost inexhaustible prodigality of Nature, it had been recognized at an early stage that the biological formation of cyclic terpenes can be traced back to the operation of a unifying and, in principle, rather simple biosynthetic scheme[5]. In the light of present knowledge three main phases can be discerned.

In the first phase (*Figure 1*), regularly built pyrophosphate esters of aliphatic terpene alcohols are formed through successive elongation of a basic
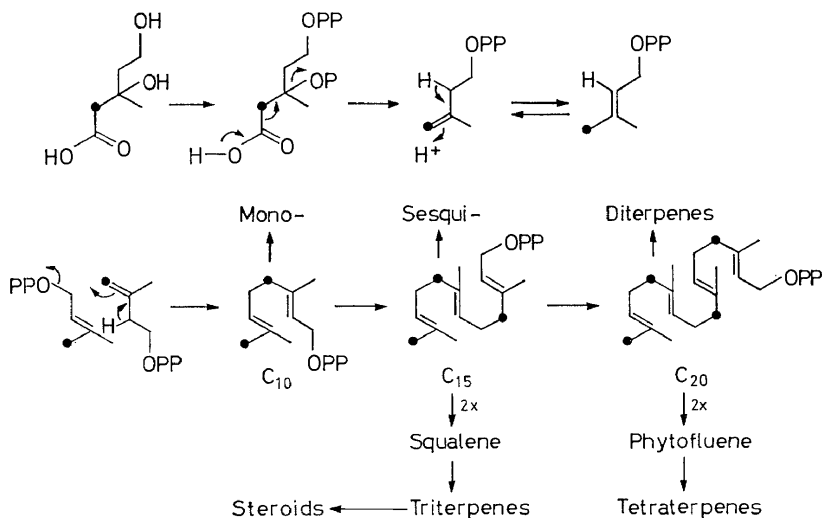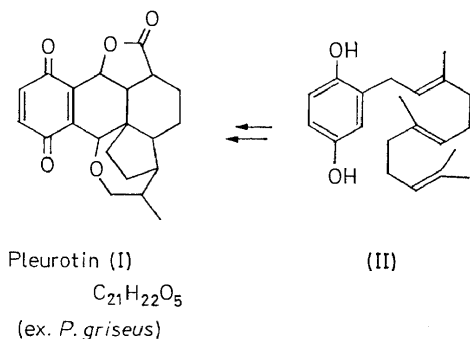

Figure 1

C_5-unit closely related to mevalonic acid. The detailed mechanism of the genesis of this important compound and of its conversion to the isoprenologous chains are by now well understood, mainly as the result of the impressive amount of work carried out at or near the enzymatic level by the groups of Bloch, Lynen, Popják and Cornforth[6]; the enzymes catalyzing these

steps seem to be universally distributed throughout living matter. Next, the aliphatic precursors are converted to cyclic terpenes through one or more cyclization steps, which may be triggered by addition of an external electrophile or by the intrinsic electrophilic nature of the allylic pyrophosphate group. The ionic intermediates so generated quite often tend to undergo more or less deep-seated rearrangements of hydrogen atoms and alkyl groups, and the process is then completed by saturation of the positive charge. In the third phase further elaboration of the cyclic prototypes can occur in a variety of secondary processes, which may include oxidation, ring cleavage, loss of side chains and even combination with units of different biosynthetic origin. Here the responsible enzymes are more specifically linked to single biological systems, and it is in fact the joint operation of the last two phases that is responsible for the astonishing structural variations encountered in Nature. A good measure of the extent to which the origin of a compound can be obscured by such transformations is provided by the structure of many indole alkaloids, which only on the basis of extended experimental work have been recently adopted as members of the terpene family[7].

Following the lines laid down by L. Ruzicka in his formulation of the biogenetic isoprene rule[5], a number of schemes at different levels of sophistication have been advanced in order to depict the genesis of cyclic terpenes[8-10]. It has become a popular game, once the structure of a new compound has been worked out, to apply the rules of comparative anatomy for a tentative biogenetic classification. Moulds and fungi have been noted in this respect as a rewarding source of surprising combinations. An illustration from our recent work (see *Figure 2*) is provided by the structure of pleurotin (I), a


Pleurotin (I)

$C_{21}H_{22}O_5$

(ex. *P. griseus*)

(II)

Figure 2

metabolite of *Pleurotus griseus* first isolated by Robbins, Kavanagh and Hervey[11]. The structure was first derived on chemical grounds[12] and eventually confirmed by an x-ray crystallographic investigation of an appropriate derivative[13]. Inspection of formula (I) suggests a mixed biogenetic derivation from an aromatic C_6- and a terpene C_{15}-unit; these could combine to farnesylhydroquinone (II) and then evolve to pleurotin through various cyclization, rearrangement and oxidation steps, the details of which are open to even further speculation.

Isolation and structural work in this field can also prove of great help in identifying missing links of hypothetical biogenetic sequences. Here again

we may quote an example from our own work (*Figure 3*). Some time ago, in cooperation with Dr. Godtfredsen and his group, we succeeded in working out the complete structure for fusidic acid (IV), a powerful antibiotic from *Fusidium coccineum*[14]. The novel stereochemistry of this compound[14, 15] suggested a biogenetic filiation from one of the non-rearranged ionic intermediates between 2,3-oxidosqualene (III) and lanosterol†, and a search for possible parent compounds was then started. This eventually led to the isolation of a new metabolite, fusisterol (V), the structure of which could be secured by chemical means[17]. As an essential part of the investigation it was found that the corresponding dihydroacetate rearranged smoothly under the influence of mineral acids to the acetate of dihydrolanosterol (VI). Of course, this chemical change is an exact duplication of part of the enzymatic process thought to be responsible for the biological formation of lanosterol, and the discovery of fusisterol opens some new and intriguing possibilities. Yet, here as in other cases, it is clear that the validity of a biogenetic scheme can ultimately be assessed only by working with living systems or with purified enzymes.

(III)

Fusidic acid (IV)

Fusisterol (V)
(ex. *F. coccineum*)

1. Ac₂O/Py
2. H₂/PdC
3. HCl/AcOH

(VI)
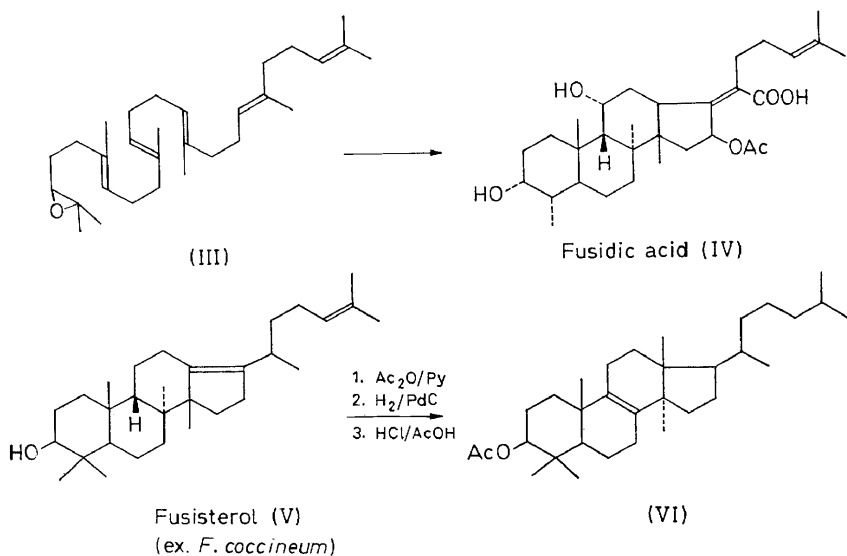
Figure 3

Some advances in this direction have been made, and the recent work of Corey, van Tamelen and their associates with oxidosqualene cyclases deserves particular mention[18,19]. But, generally speaking, for the polycyclic terpenes progress in this area has been comparatively slow. Inefficient effort may well be one of the reasons, but other factors must be taken into account. Many of the biological systems investigated, especially among higher plants, have displayed so far a regrettable lack of cooperation; only a limited number of building blocks are available, most of them are difficult to handle on a small scale and raise formidable problems of permeability; finally, the

† Conversion of 2,3-oxidosqualene to fusidic acid has been reported recently[16].

cyclization step itself, which is the most dramatic expression of terpene biosynthesis, is by its very nature rather elusive. In the best known of these processes, the conversion of 2,3-oxidosqualene to lanosterol, four C—C bonds are formed, two hydrogen atoms and two methyl groups undergo specific rearrangements, and not less than six chiral centres are created; yet no stable intermediates have been detected so far, and it seems that, if formed at all, they are likely to be covalently bound to the enzyme. This situation is in sharp contrast with the one that prevails for alkaloid bio-synthesis, where a plethora of intermediates interrelated by simple steps can usually be detected, much to the benefit of the workers in this field.

In an attempt to fill some of the existing gaps, we decided to explore the detailed biosynthesis of at least one representative terpene not related to the lanosterol family. In the following I shall use our approach to illustrate in a specific case the problems one is confronted with in this field and the sort of solutions which can nowadays be provided.

As the objective of our efforts we choose pleuromutilin (VII), a metabolite from *Pl. mutilus* which had held our interest for some time and which provides yet another proof for the chemical versatility of this family of fungi. The struc-ture of pleuromutilin (*Figure 4*) rests on firm chemical grounds[20-21], the stereochemistry of all eight chiral centres has been defined rigorously[22] and the overall picture has been verified independently by x-ray analysis[23] of

R = —COCH2OH   Pleuromutilin (VII)
R = —H   Mutilin (VIII)

(ex. *Pleurotus mutilus*)
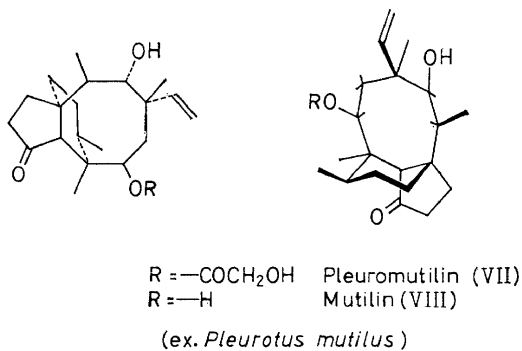
Figure 4

the monobromoacetate of mutilin (VIII), the parent C_{20}-compound. In addition, the crystallographic work has revealed that the eight-membered ring adopts, at least in the crystal, a conformation which is practically identical with that of simple cyclooctane derivatives. The selection of this specific compound seemed justified for a number of reasons: to begin with, the unusual and intricate carbon skeleton itself, including the rather rare eight-membered ring, warranted a biosynthetic investigation; secondly, the antibiotic is produced in good and reproducible yields in submersed cultures under standardized conditions; last but not least, the substitution pattern of the molecule was such as to permit detection of label at almost any wanted position.

As a guiding line for the experiments to be performed, a hypothetical scheme was set up[24] which allows one to account for the formation of pleuro-

mutilin in the light of the general postulates (*Figure 5*). Cyclization of the suspected aliphatic precursor, the pyrophosphate of all-*trans* geranylgeraniol (IX) is initiated by addition of an external proton, and leads to a bicyclic ionic intermediate (X); next, the positive charge induces the successive migration of a hydrogen atom, a methyl group and than a second hydrogen atom. The next stage is the somewhat unusual contraction of a six- to a five-membered ring, following which the process comes to a first stop by extrusion of a proton from one of the two geminal methyl groups. It is worth mentioning at this point that the two methyl groups are neither sterically nor biogenetically equivalent, and that theoretical considerations do not allow one to decide whether the proton elimination will occur in a stereospecific manner and, if so, which of the two methyl groups will be involved. When the formula of the bicyclic intermediate (XIa) is rewritten in an appropriate manner as in (XIb), it is easily seen that solvolysis of the allylic pyrophosphate group can now start an electrophilic attack on the double bond of the iso-pentenyl chain. The tricyclic carbonium ion (XII) thus generated possesses the required carbon skeleton, and formation of the antibiotic is then completed by saturation of the positive charge (a process which will be considered in detail at a later stage), secondary oxidation steps and introduction of the glycolic acid unit.
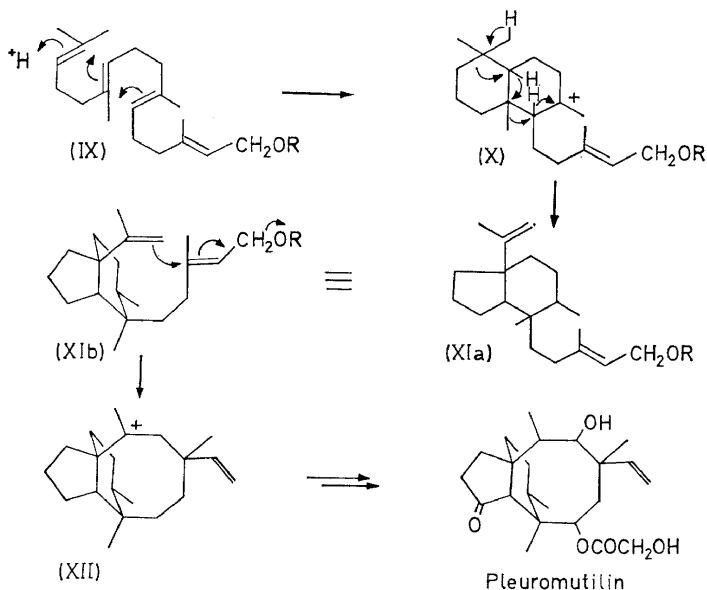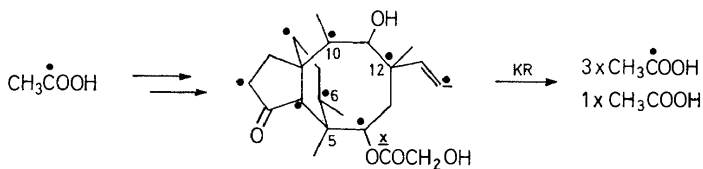

Figure 5

This scheme contains a number of detailed predictions amenable to experimental verification.† In preliminary experiments we investigated the incorporation of carboxyl labelled acetic acid. The expected pattern of label is indicated in *Figure 6* by dots, and ozonolysis of the hydrolysed material proved that the expected amount of radioactivity was indeed located in the terminal

† Some independent work on the biosynthesis of pleuromutilin has been carried out by Birch, Holzapfel and Rickards.[21]

methylene group. As a consequence of the postulated backbone rearrangement, the methyl group at C-5 has been shifted from a carboxyl to a methyl-derived carbon atom; degradation of the acetic acid obtained upon Kuhn–Roth oxidation of a selected derivative gave results consistent with the idea that one equivalent out of four is free from radioactivity. Finally, degradation of the glycolic acid residue indicated that some label was specifically incorporated into the carboxyl group, the ratio with respect to a labelled carbon of the nucleus being 0·66. This result, including the observed dilution, is best interpreted as a consequence of the joint operation of the citric and glyoxalic acid cycles and proved of some importance in the later developments.

Figure 6

Clear-cut evidence for the terpene nature of pleuromutilin was obtained upon feeding of 2-^{14}C-mevalonate (*Figure 7*). In this experiment the glycolic acid residue was found to be radio-inactive and the label was confined to the C_{20}-component. For the required degradations we could now rely on the experience gained in the course of the structural work, although occasionally new procedures had to be devised. C-3 was carved out as benzoic acid by Kuhn–Roth oxidation of the corresponding phenylcarbinol (XIII), C-10 and the attached methyl group were eliminated by transformation to the
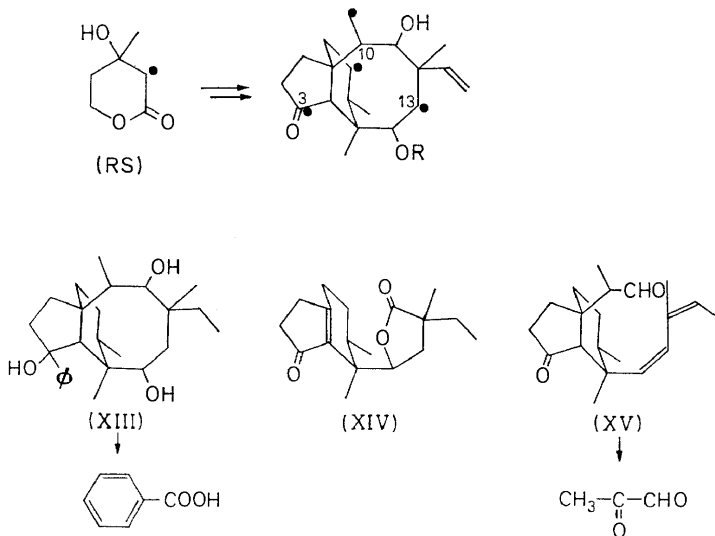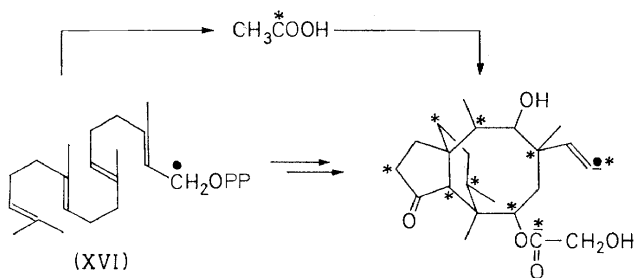

Figure 7

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known bisnor-lactone (XIV), and pyrolysis of the antibiotic to the diene (XV), followed by ozonolysis to methylglyoxal and stepwise degradation of the latter, permitted isolation of C-13. In this manner each of the three atoms was shown to carry one quarter of the original label. As indicated before, location of a full label at the C-10 methyl group, rather than at C-11, could not have been predicted by the scheme; the result is of some importance since it proves that the elimination of a proton, which concludes the first cyclization, is subjected to stringent steric requirements by the enzyme involved.

Having thus gained some confidence in the validity of our scheme we could now proceed to a closer examination of one of its crucial tenets, namely the direct involvement of the aliphatic C_{20}-precursor. Here a number of problems had to be solved. We elected to label C-1 of the aliphatic chain (see *Figure 8*) since this allowed for a subsequent easy isolation of the radio-active label. Synthesis of the desired compound (XVI) was readily accomplished by a Reformatzky reaction between farnesylacetone and 1-^{14}C-


	PM (VII)	Glycolic acid HOOC$\dotplus$CH$_2$OH	M (VIII)	C_{19}^- aldehyde	CH$_2$O
Disrupted cells	—	—	—	100	25	65
Whole cells	100	3·5	<0·8	86	51	35

Figure 8

labelled bromoacetic ester, followed by dehydration and reduction to a mixture of *trans* and *cis* tetraprenols in an approximate ratio of 4:1; this mixture was then converted by known methods to a crude preparation of the corresponding pyrophosphates. Because of its pronounced polar character, such a precursor could hardly be expected to permeate efficiently the cell membrane, though at least one apparent exception has been recorded[25]. Accordingly we deemed it wise to modify our biological system. After a number of false starts it was found that suitable preparations could be obtained by brief treatment of the washed mycelium with ultrasound. A 24 hours incubation was then started with the labelled material in the presence of suitable cofactors, while a parallel experiment was run with intact cells. Following dilution with inactive carrier material and rigorous purification, it was rewarding to find that some radioactivity had been incorporated in both runs, albeit in rather modest yields. This, however,

takes the argument only part of the way, and it was mandatory to pin down the exact location of the label; as indicated in *Figure 8* ozonolysis of mutilin from the first experiment gave formaldehyde containing 65 per cent of the original label, and 25 per cent residual activity in the C_{19}-aldehyde. These figures are quite satisfactory inasmuch as they vindicate the contention that a major amount of radioactivity is incorporated from an intact precursor in accordance with the scheme. But how should the substantial amount of residual radioactivity in the C_{19}-aldehyde be accounted for? A clue to the problem came from the ozonolysis of radioactive pleuromutilin from the parallel run with intact cells: in this case, not only the ratio of the two fragments had decreased to 35:51, but the availability of glycolic acid also enabled us to detect that 3–4 per cent of the label had been incorporated rather specifically into its carboxyl group. Bearing in mind the result of a previous experiment, one is led to the surprising conclusion that in both runs part of the radioactivity has been incorporated via carboxyl-labelled acetic acid, and more pronouncedly so in the experiment with intact cells. While the exact mechanism of this unexpected and rapid breakdown of the C_{20}-precursors remains unsettled, it is tempting to speculate that the path followed here is similar to the one discovered by Seubert[26] for the utilization of geraniol and related compounds in *Pseudomonas citronelloli*.

Rapid catabolism of aliphatic terpenes seems a normal reaction even in higher plants. In connection with current work on the biosynthesis of indole alkaloids we have had occasion[27] to feed methyl-labelled geraniol (XVII) as the free alcohol to rhyzomes of *Menyanthes trifoliata* (*Figure 9*). Subsequent degradation of the terpene glucosides loganin (XVIII) and foliamenthin
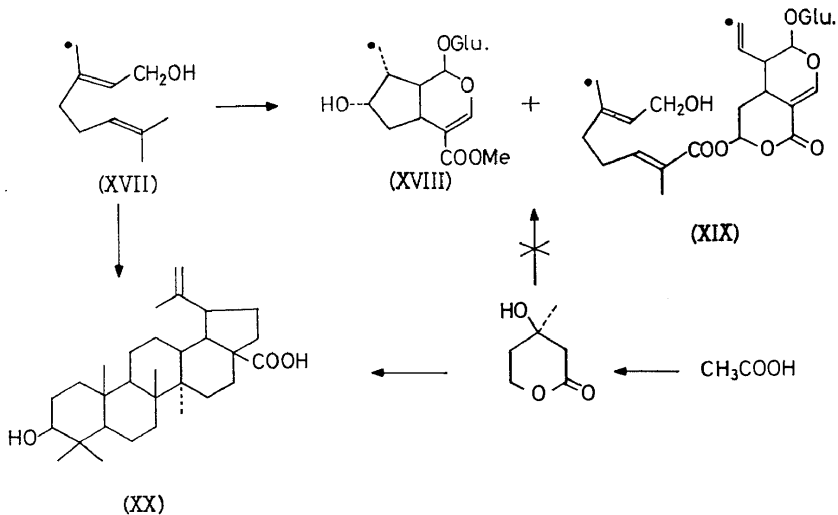
Figure 9

(XIX)[28] revealed that in both substances the label was restricted to the expected position. We failed to realize how lucky we had been until a sample of betulic acid (XX) from the same plant specimen was analyzed and found to be radioactive[29]. Further studies indicated that the label had been

introduced mainly from small fragments and only to a minor extent by direct incorporation, and, moreover, that in this plant acetic and mevalonic acids behave as efficient precursors for the triterpenes and the phytosterols but not for the monoterpene glucosides. Clearly, many of these experimental pitfalls would be eluded by working with purified enzymes. As long as this goal remains unattained, the important lesson of such results is that incorporation (or lack of incorporation) of radioactivity *per se* may turn out to be very misleading, and that exact location of the label by appropriate chemical manipulation is an essential part of biosynthetic studies.

We may now return to our main theme and consider in more detail the matter of the two 1,2-rearrangements of hydride ions linked up with the first cyclization step. Evidence for the migration of a methyl group has been mentioned already. Inspection of the scheme in *Figure 5* indicates that by a lucky coincidence both migrating H-atoms were linked originally to C-4 of mevalonic acid, and a correspondingly tritiated specimen is therefore the precursor of choice for the planned experiment. From the elegant work of Popják, Cornforth and their coworkers[30] it is known that the two diastereotopic H-atoms in the C-4 methylene group of mevalonic acid suffer different fates on the way to the polyprenol chain, and that, as indicated in *Figure 10*, H_A is specifically retained in the formation of *trans* double bonds and specifically lost when *cis* double bonds (e.g. as in rubber) are formed. To gain
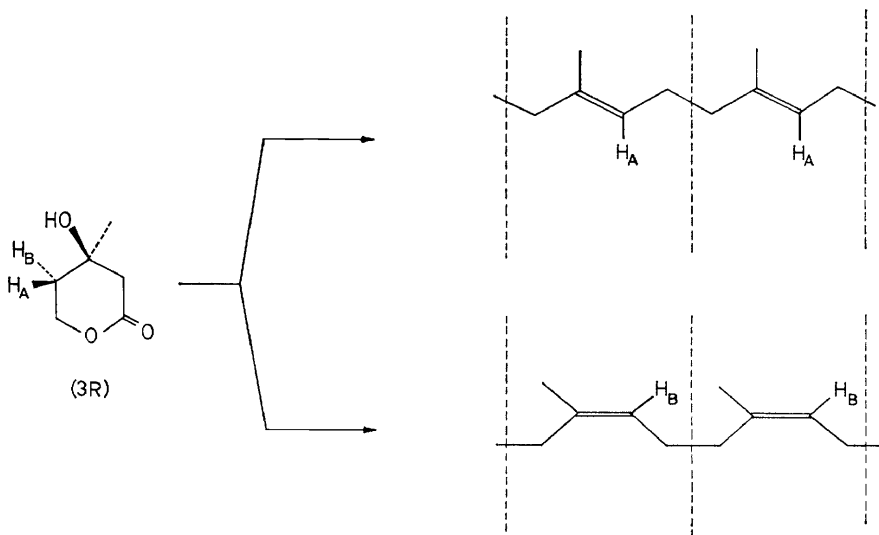

Figure 10

additional information an incubation was therefore carried out with sodium (3R,4R)-4-³H-mevalonate, used in admixture with its optical antipode. The problems which arise from the use of a racemic precursor need not bother us in this connection, as we shall return to the point later on. The recovered pleuromutilin (see *Figure 11*) was found by degradation to contain exactly four equivalents of tritium. Two of these were easily located at C-4 and in the side chain by enolization of the C-3 carbonyl group respectively
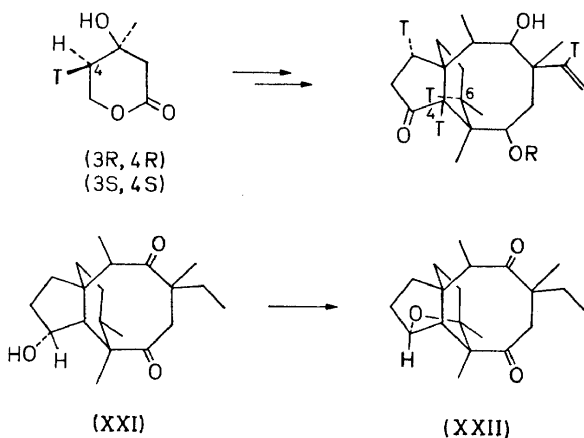
Figure 11

by stepwise degradation of the vinyl group, first to the aldehyde and then to the corresponding acid. The tritium at C-6, which is critical for our demonstration, is embedded in a rather unreactive part of the molecule; its detection was made possible by oxidation of the diketoalcohol (XXI) with lead tetraacetate, which gave a very good yield of the cyclic ether (XXII). Besides verifying the postulated double 1,2-migration of H-atoms, these results also prove that all four double bonds in the immediate aliphatic precursor are indeed of the *trans* type and that the stereochemistry of pleuromutilin both at C-4 and C-6 is genuine in a biosynthetic sense rather than due to subsequent epimerizations. On this basis the stereochemistry of the first cyclization step deserves some comment. The *cis* arrangement of the hydrogen atom at C-6 and the methyl group at C-5 on the six-membered ring of the antibiotic requires a *syn* relationship of the same atoms prior to the rearrangement in the bicyclic ionic intermediate (XXV); *Figure 12* shows that this stereochemical situation can be arrived at either by chair–chair cyclization of a
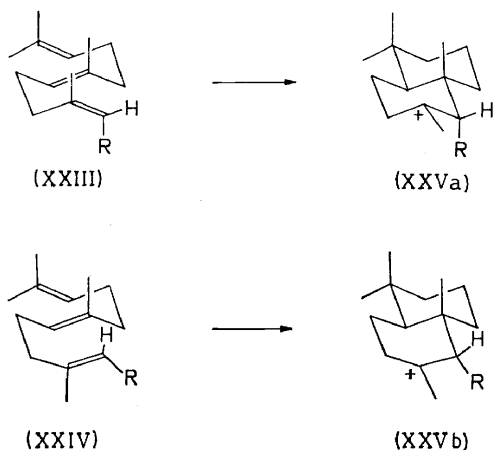

Figure 12

trans–cis precursor (XXIII) → (XXVa), *or* by chair-boat cyclization of a *trans–trans* precursor, (XXIV) → (XXVb). The available evidence is in agreement only with the second possibility, and the situation is therefore exactly like the one which has been discussed[8], but never proved, for the corresponding chain segments in the conversion of squalene to lanosterol. The topography of the ensuing rearrangement is also of considerable interest. It can be seen from *Figure 13* that, following migration of the H-atom, the methyl group moves to the unsaturated centre from the same side of the ring-plane; this rules out a fully concerted process. Stabilization by loss of a proton from the adjacent methylene group followed by a new electrophilic attack is excluded by the tracer work discussed below. Among the remaining possibilities, transient reversible addition of some nucleophile, perhaps a basic group of the enzyme surface, is an obvious one, which would restore the desired antiplanar character of the process.
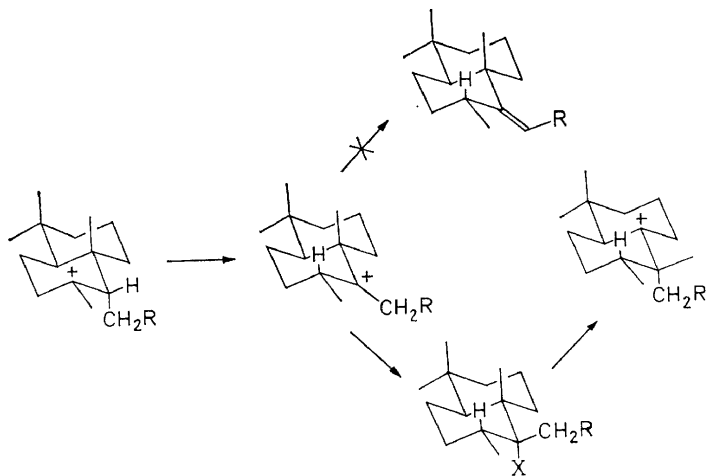

Figure 13

So far no attention has been paid to the ways in which the tricyclic ion from the second cyclization step disposes of its charge. A fortuitous observation made in the course of the structural work played an important role in this connection (*Figure 14*). Briefly, it was found that upon treatment with phosphoroxychloride in pyridine the monoacetate (XXVI) was smoothly transformed into an anhydro derivative, the structure of which is firmly established as (XXVII); it follows that the dehydration process must include a very facile 1,5-transannular migration of a hydride ion from C-10 to C-14. The distance between the two carbon atoms is very favourable, as confirmed by the x-ray work[23], and the great ease with which the reaction occurred suggested that a similar migration in the opposite direction, (XXVIII) → (XXIX), might well be involved in the biosynthetic sequence. Addition of a nucleophile to the new unsaturated centre is expected to take place with inversion of configuration at the relevant carbon atom, and the resulting scheme has the additional merit of assigning to the C-14 oxygen function of the antibiotic the precise role of a biogenetic marker. The first

part of our views was tested in experiments with 5-^{3}H$_2$-mevalonate (*Figure 15*). Of course, it must be realized that only very few molecules of this substrate are actually double labelled and the symbol we use is simply meant to signify that the label is evenly divided between the two disatereotopic positions. Following incorporation, it was an easy matter to locate two equivalents of tritium each at C-2 by enolization and in the terminal methylene
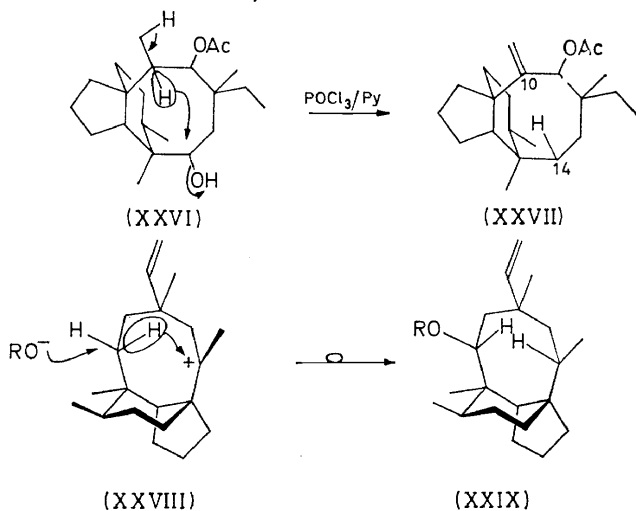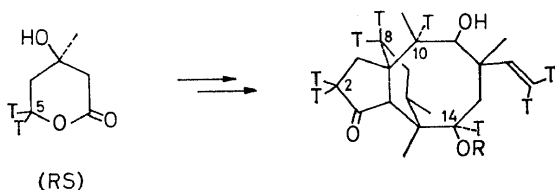

Figure 14

Figure 15

group by ozonolysis. One further equivalent was shown by specific oxidation procedures to reside at C-14, and the *bisnor-seco* compound (XXX) available by elimination of the vinyl chain and *retro*-Michael cleavage of the eight-membered ring, helped in detecting the presence of two additional tritium atoms at C-8. While this general picture was quite satisfactory, a more direct proof for the crucial presence of tritium at C-10 was considered mandatory.

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At first glance one might think that enolization of a suitable C-11 oxo-derivative would serve the purpose, but the actual situation proved to be more complex. As indicated in *Figure 16* for the diketone (XXXI), the eight-membered ring is locked in a conformation which avoids unfavourable interactions, and in this conformation it is impossible to accommodate enolate double bonds at the crossed positions. This is dramatically certified by the behaviour of (XXXI), where the three hydrogen atoms adjacent to the carbonyl groups cannot be exchanged, even under forcing conditions. In the event, a solution to the problem was found in the base-catalyzed conversion of the suitable modified derivative (XXXII) to the tetracyclic compound (XXXIII) which, as expected, behaved quite normally under enolizing conditions. In this way, it could be shown conclusively that in the labelled material exactly $\frac{1}{8}$ of the total activity resided in the C-10 methine group.
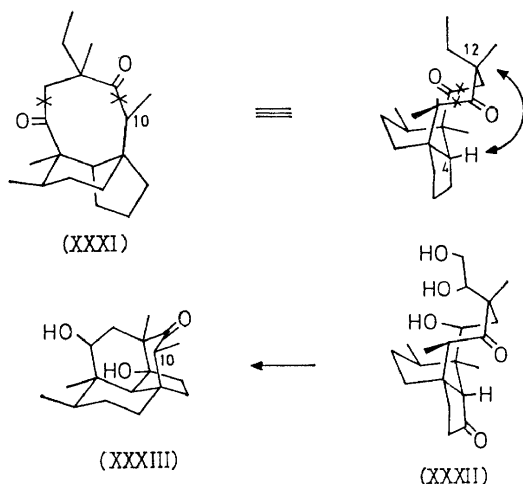

Figure 16

Having established the transannular migration, we could now approach the stereochemical problem posed by the subsequent addition of the nucleophile. In principle, the question of retention versus inversion at C-14 can be answered by using mevalonic acid stereospecifically labelled at C-5. Such specimens have already been prepared before[31], but their stereochemistry requires some comment. As indicated in *Figure 17* when racemic mevaldic acid is reduced with NAD-T in the presence of the appropriate enzyme, both enantiomers react to afford what in all probability is a mixture of two diastereoisomers. One of these can be transformed to farnesol by a preparation of rat liver known to be selective for the (3R)-isomer of mevalo-lactone, and the stereochemistry of the carbinol group, corresponding to C-5 of the starting material, can be characterized by the observation that all of its label is removed upon stereospecific oxidation with liver alcohol dehydrogenase. Originally, the stereochemistry of the chiral centre was inferred by an indirect method which relies on partial asymmetric synthesis[32]. We have now verified this assignment for the similarly behaving geraniol by a direct correlation. To this purpose, the stereospecifically labelled

geraniol was first converted to the benzyl ether and then degraded to ethanol by the straightforward sequence indicated in *Figure 18*. The stereochemistry of the tritiated ethanol was assayed enzymatically with liver alcohol dehydrogenase (LAD) after calibration of the enzyme with an authentic sample of known configuration[33, 34], and found to be as indicated. This, in turn, settles the configuration of the geraniol and hence of the starting mevalonic acid as (3R,5R).
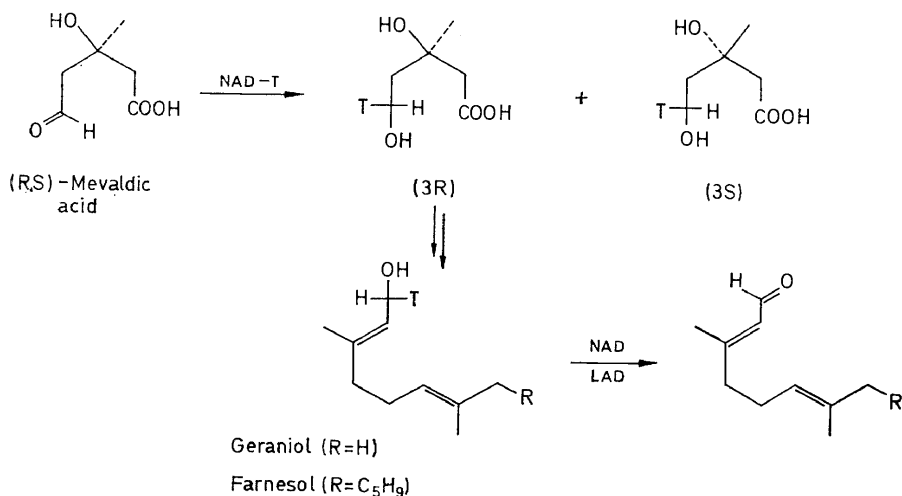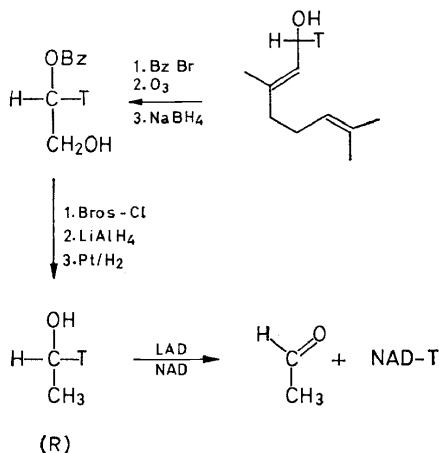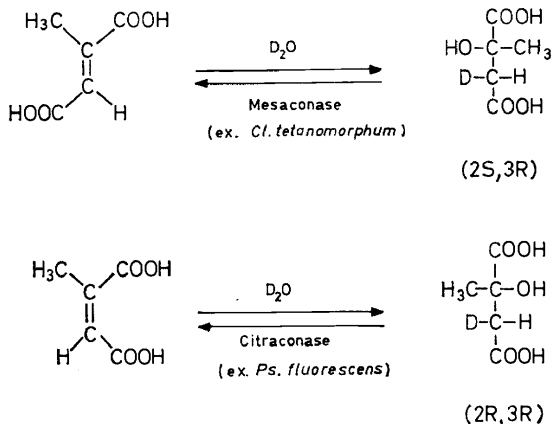

Figure 17

Figure 18

Though it is usually taken for granted that it is always the same optical form of mevalolactone that is universally used for terpene biosynthesis, the evidence for this is rather meagre, and the recent work of Gottschalk and Barker[35] on variations in the stereochemistry of citric acid formation in different biological systems indicates that great caution should be exerted

in making such generalizations. For this reason we felt it necessary to make sure that it was indeed the (3R)-isomer of mevalolactone rather than its optical counterpart which was partaking in our biosynthetic sequence. Optical resolution of mevalonic acid being hardly practicable at the scale at which labelled substrates are usually employed, we had to resort to some other device. In this we were greatly helped by work carried out in our laboratory on the enzymatic formation of citramalic acid[36, 37] (see *Figure 19*). Each of the two optically pure isomers of this acid can be generated in quantity from mesaconic and citronic acid respectively through the action of known enzymes[38, 39]; in both cases the reaction also allows for stereo-specific introduction of label at the methylene group.

Figure 19

The conversion of citramalic acid to mevalolactone is illustrated in *Figure 20*. The (2S)-isomer (XXXIV) was reduced as the dimethylester to a triol (XXXV), which could be converted via (XXXVI) to the monotosylate (XXXVII). Reaction of the latter with ^{14}C-labelled potassium cyanide gave the nitrile (XXXVIII), which, in turn, could be hydrolysed directly to (3S)-1-^{14}C-mevalolactone. On the other hand a sequence of oxidation and catalytic reduction led from the nitrile (XXXVIII) to the amino acid (XXXIX), which, upon deamination, provided an entry to (3R)-mevalolactone labelled at C-5. The scheme is quite flexible, and it is clear that by a judicious combination with the aforementioned enzymatic reactions it permits the preparation of a variety of optically pure mevalolactones labelled with a given isotope at specific positions. This is illustrated in *Figure 20* for a deuterium label.

Working along these lines, it was now an easy matter to show that our fungus was indeed incorporating the (3R)-isomer of mevalolactone. With these hard-won facts in hand we could then proceed to the crucial experiment (*Figure 21*). Starting with (3R,5R)-5-[3]H$_1$-mevalolactone and bearing in mind the tacit assumption that, as in squalene biosynthesis[30], the building up of the chain implies for each elongation step a formal inversion of configuration at C-5 of the precursor, the stereochemistry of the tricyclic ion can be predicted as in (XL). Now it is the hydrogen which undergoes the
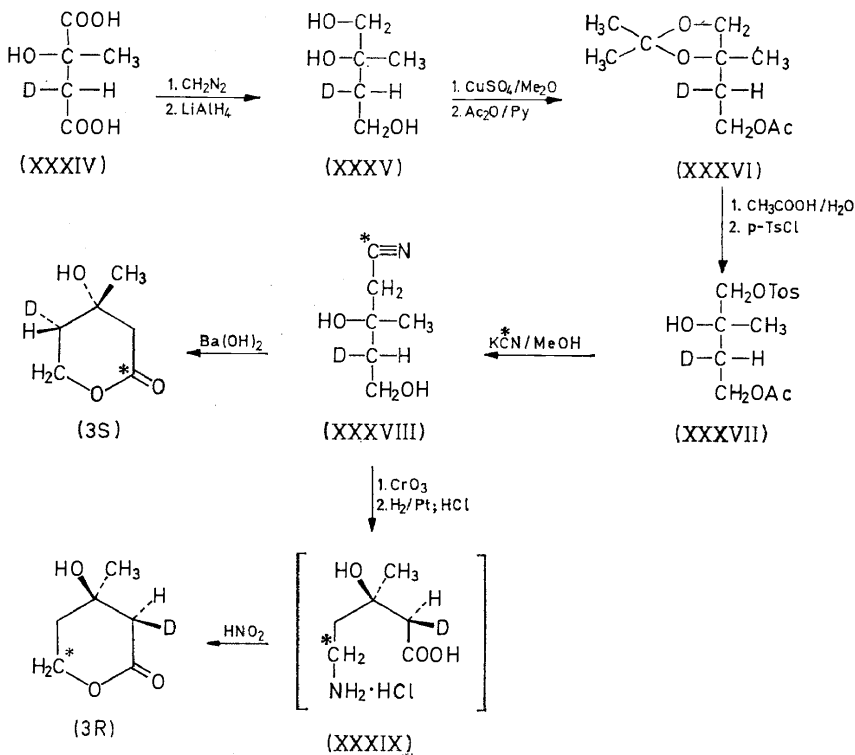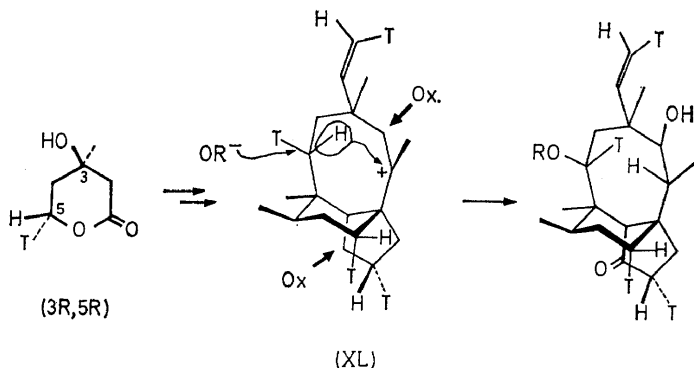
Figure 20

Figure 21

transannular shift, and as a consequence of the new inversion caused by the addition of the nucleophile, the tritium atom takes up the inside position in the product. Degradation of the labelled material along the lines delineated before confirmed that this is indeed what happens.

So as to make the story complete and check the validity of the underlying assumption, namely inversion of configuration in the elongation step, the stereochemistry of the label at C-2 was also established (*Figure 22*). To this

purpose the diketo-alcohol (XLI) was benzoylated and then pyrolyzed to give a substance (XLII) that differed from the dehydration product (XLIII), available from (XLI) on treatment with phosphoroxychloride in pyridine. Both (XLII) and (XLIII) retained all of the activity of the precursor (XLI), and this, together with the well-known *cis* mechanism of pyrolytic reactions, fully defines the stereochemical situation at C-2. We conclude that the mechanism for the chain extension in the formation of pleuromutilin follows the same steric course as in the biosynthesis of squalene with rat liver enzymes. Moreover, introduction of the oxygen function at C-14 in the antibiotic occurs with inversion of configuration and represents the last step of the second cyclization-rearrangement sequence.
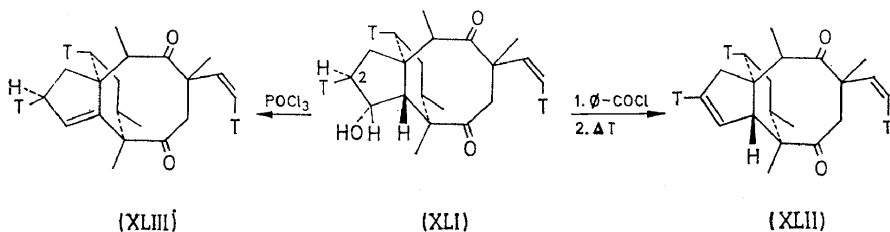

Figure 22

This, then, is the actual state of our knowledge of the biosynthesis of pleuromutilin. As so often in biosynthetic investigations, a number of mechanistic problems remain to be solved, but it is clear that, here and elsewhere in this area, substantial progress will require a closer study of the *deus ex machina* of terpene biosynthesis, the cyclization enzyme itself.

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