285. *Studies in Relation to Biosynthesis. Part XXIX.* The Terpenoid Chain of Mycelianamide.*

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Methylgeraniolene (VIII; R = H) was obtained from mycelianamide labelled biosynthetically by feeding [2-^{14}C]mevalonic lactone to *Penicillium griseofulvum*. It was oxidised, by feeding it to a rabbit, into Hildebrandt's acid which is shown to have the stereochemistry and chiefly the labelling pattern of (IX). Therefore the labelling pattern of the methylgeraniolene is probably that in (VIII; R = H), in accord with expectation.

The biosynthesis of terpenoid compounds involves [1] the conversion of mevalonic acid (I) successively into the isomeric isopentenyl pyrophosphates (II) and (III), which then polymerise into terpene chains of varying length [*e.g.*, geranyl pyrophosphate (IV)] by reactions of the type (A). With [^{14}C] in the 2-position of mevalonic acid (I) the expected labelling patterns of compounds (II) and (III) are those shown; for (III) it is difficult to devise methods to investigate this pattern directly, since the difference between the methyl groups is merely steric. Indirect evidence of non-randomisation of the label between them, that is, of complete stereospecificity of the conversion (II) ⟶ (III), has come from investigations of cyclic terpenoid compounds where the two methyl groups have been distinguished from each other, usually by specific biological oxidation but in the case of trichothecin by migration.[2] The compounds investigated include rosenonolactone (V) and gibberellic acid,[3] soysapogenol-A,[4] elymoclavine (VI; R = OH) and agroclavine (VI; R = H).[5] The first three compounds involve a concerted stereospecific cyclisation of a polyterpene chain, ring A being generated as shown. The *gem*-dimethyl groups originally present at the end of the chain are distinguished from each other at some stage by biochemical oxidation, and that one which becomes equatorial to ring A after cyclisation (compare V) is found to be labelled when the substance is derived from [2-^{14}C]mevalonic lactone [*i.e.*, from (II) and (III)]; the oxidised methyl group, present as the lactone carbonyl in (V), is unlabelled.[4,5] Similarly, in compound (VI) the activity from [2-^{14}C]-mevalonic lactone appears mainly in the CH_2R group.[5] In both types of compound the

* Part XXVIII, *J.*, 1962, 421.

[1] See, *e.g.*, Lynen and Henning, *Angew. Chem.*, 1960, **72**, 820; Popjak and Cornforth, *Adv. Enzymol.*, 1960, **22**, 287.

[2] Jones and Lowe, *J.*, 1960, 3959.

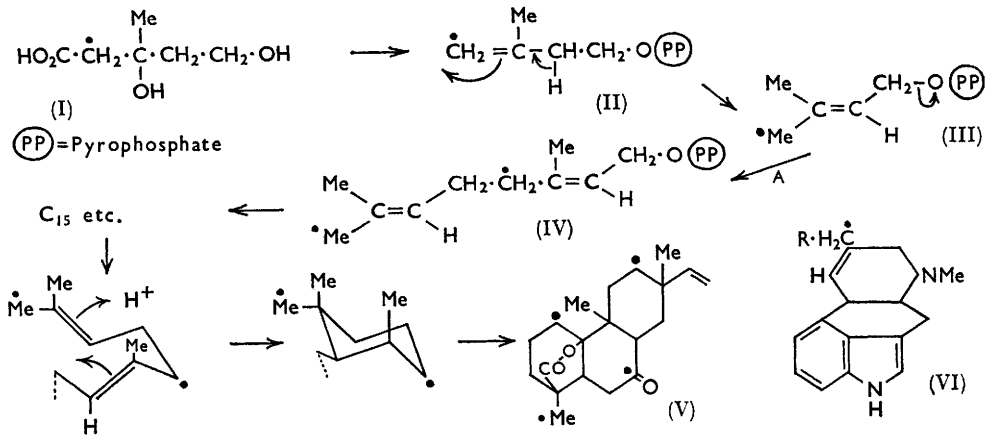
[3] Birch, Rickards, Smith, Harris, and Whalley, *Tetrahedron*, 1959, **7**, 241; Britt and Arigoni, *Proc. Chem. Soc.*, 1958, 224.

[4] Arigoni, *Experientia*, 1958, **14**, 153.

[5] Bhattacharji, Birch, Brack, Hofmann, Kobel, Smith, and Winter, *J.*, 1962, 425.

evidence leads to the deduction that the labelling pattern of the original terminal unit is that shown in (III). However, in view of the importance of this conclusion in considering the mechanisms of such cyclisations and oxidations we have obtained further evidence from an open-chain terpene.

It is difficult to examine an open-chain compound for several reasons. There are few such compounds into which incorporation of the precursor could at present be achieved

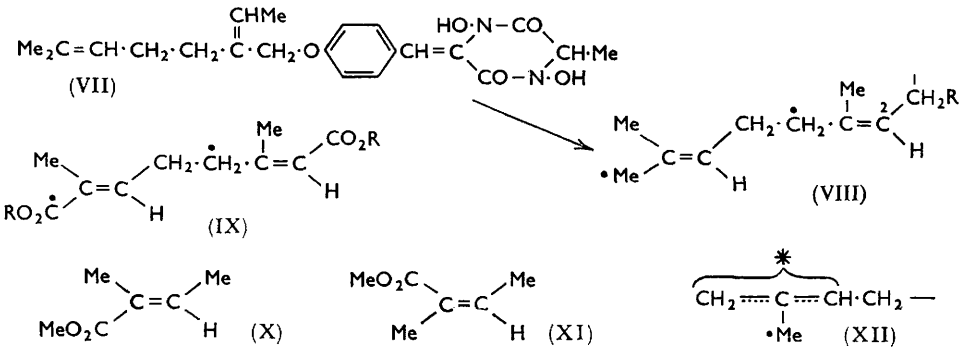


to a useful extent: two examples are squalene and mycelianamide (VII). Further, although allylic substitutions might be developed to distinguish the sterically different methyl groups in the *gem*-dimethyl group, none is at present obviously applicable. Mycelianamide (VII), produced by a strain of *Penicillium griseofulvum*, has been reduced [6] by sodium in liquid ammonia to yield methylgeraniolene (VIII; R = H), which should have the labelling pattern shown when [2-^{14}C]mevalonic lactone is the biosynthetic precursor. After it has been fed to rabbits methylgeraniolene is excreted in the urine in the form of Hildebrandt's acid (IX; R = H),[7] the stereochemistry of which was incompletely known. It appears, however, to consist of only one isomer and, since it is also produced by the metabolism of citral or geraniol (VIII; R = OH) the known steric disposition about the 2-double bond is probably retained in the acid (IX). The problem is, therefore, to determine the stereochemistry of the other double bond if use is to be made of the biochemical oxidation to examine the labelling pattern. From the mode of synthesis [8] it appears that Hildebrandt's acid is probably the most thermodynamically stable isomer, but this is by no means certain.

This stereochemistry has now been defined by proton magnetic resonance. The spectrum of the dimethyl ester (IX; R = Me) has =CH– peaks at τ values [9] of 4·30 and 3·30. The former is fairly normal and must arise from a group =CH·CO_2Me; the latter is unusually low, suggesting that the other =CH– is located *cis* to the other CO_2Me group.[10,11] Earlier determinations of *cis–trans*-isomerism by this method have usually been made with both isomers available, but the characterisation of the single isomer is probably reliable in this case as the τ value 3·30 is very close to that of compound (X) (3·28) and differs considerably from that of the isomeric ester (XI) (4·03).[10] There is little likelihood of the resonance at 3·30 corresponding to =CH·CO_2Me, even though this =CH– is

[6] Birch, Massy-Westropp, and Rickards, *J.*, 1956, 3717; Birch, English, Massy-Westropp, and Smith, *J.*, 1958, 369.
[7] Kuhn, Köhler, and Köhler, *Z. physiol. Chem.*, 1936, **242**, 171.
[8] Kuhn and Grundmann, *Ber.*, 1937, **70**, 1894.
[9] Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1960.
[10] Cf. Nair and Adams, *J. Amer. Chem. Soc.*, 1960, **82**, 3786.

α to CO_2Me, since even in dimethyl maleate [11] the analogous peak is at 3·86; the experimental τ value of 4·30 seems reasonable for this =CH− group. The resonance at 3·30 is also the broader of the two, as expected because of the additional coupling with the adjacent allylic $-CH_2-$. The two values corresponding to $H_3C{\cdot}C{=}C$ (7·80 and 8·18) agree with the stereochemistry in formula (IX). The former clearly corresponds to the methyl



group which is *cis* to the right-hand CO_2Me in (IX; R = Me) (cf. ref. 11), proving that, as expected, the geraniol–geranic acid stereochemistry is retained. Each of these methyl resonances shows signs of doublet fine structure caused by long-range coupling with the =CH− protons.

Decarboxylation by Schmidt degradation [12] of Hildebrandt's acid (IX; R = H) (r.m.a. $32{\cdot}7 \times 10^3$) gave barium carbonate (1·24 mol., r.m.a. $6{\cdot}68 \times 10^3$); the calculated r.m.a. on the basis of the distribution in (IX) is $8{\cdot}19 \times 10^3$, if equal contributions from each carboxyl are assumed. Kuhn–Roth oxidation of the acid (IX; R = H) gave acetic acid (1·8 mol.) which had low activity (4-phenylphenacyl ester, r.m.a. $1{\cdot}35 \times 10^3$); Schmidt degradation of the acetic acid gave inactive carbon dioxide, so that all of the radioactivity present is on the methyl group. From these results, and previous degradations of methylgeraniolene derived from [2-^{14}C]mevalonic acid,[6] it appears that the major distribution of activity in Hildebrandt's acid is that in (IX), but that the terminal methyl group contains about one-fifth of the activity of the carboxyl group. This can be explained by some lack of steric specificity during the biochemical oxidation of the methylgeraniolene; an intermediate radical such as (XII) would give an opportunity for the required inversion of the stereochemistry. Although the configuration of allylic radicals is usually retained, there is a strong possibility that *cis*-compounds in particular structures and under the correct conditions can become inverted to a considerable extent; [13] in the present case only a small proportion of inversion would be required and only the resulting *trans*-acid is isolated.

The situation is therefore rather similar to that found with elymoclavine (VI; R = OH) and agroclavine (VI; R = H) [5] where labelling is found chiefly on the CH_2R group *cis* to the hydrogen atom on the double bond of the terpene unit, with a minor proportion of labelling on the $-CH_2-$ group next to nitrogen. Presumably the ring-closure on to nitrogen is preceded by some similar allylic oxidation.

All the previous evidence on the biosynthesis of terpenoid compounds suggests absence of randomisation during linkage of the isoprene units; the present evidence, therefore, strongly indicates, although it does not unequivocally prove, the labelling distribution (VIII) for methylgeraniolene derived from [2-^{14}C]mevalonic acid.

[11] Jackman and Wiley, *J.*, 1960, 2886.
[12] Cf. Wolff, *Org. Reactions*, 1946, **3**, 307.
[13] Walling and Thaler, *J. Amer. Chem. Soc.*, 1961, **83**, 3877.

Experimental

The apparatus and conventions were those used in previous Parts of this series; samples were measured on a 0·3 cm.^{2} planchette.

The proton nuclear magnetic resonance spectra were obtained at 40 Mc./sec. by using a Varian V4300 B spectrometer and 12″ electromagnet with flux stabilisation and sample spinning. Spectra were measured in saturated solution in chloroform at room temperature and are quoted on the τ scale. Peaks were measured relative to tetramethylsilane as an internal standard,[9] with side-bands generated by a Muirhead–Wigan D695 A decade oscillator.

Growth, extraction, and reduction of mycelianamide were as previously described.[6] The methylgeraniolene from mycelianamide (4·63 g.) (r.m.a. $126 \pm 1 \times 10^3$) was diluted to 4·62 g. with substance from the reduction of inactive linaloöl.[14] It was fed to a male rabbit (3 kg.) by stomach tube in two portions on successive days, and the urine was collected to a total of 4 days. The total urine (190 c.c.) was made alkaline, filtered, extracted with ether, re-acidified, and continuously extracted with ether for 2 days. The residual gum, taken up in a little ethyl acetate, was seeded and the crystals (71 mg.) were collected, sublimed at *ca.* 0·01 mm., diluted to 110 mg. with pure material, and crystallised to constant activity. This acid (17 mg.; r.m.a. $32{\cdot}75 \pm 0{\cdot}3 \times 10^3$) was decarboxylated by Schmidt's method [12] and the carbon dioxide (1·3 mol.) collected as barium carbonate (r.m.a. $6{\cdot}68 \pm 0{\cdot}2 \times 10^3$; calc., $8{\cdot}17 \times 10^3$). Kuhn–Roth oxidation of the acid (25 mg.; r.m.a. $32{\cdot}75 \pm 0{\cdot}3 \times 10^3$) gave acetic acid (1·8 mol.), converted into the 4-phenylphenacyl ester, m. p. 113° (uncorrected) (r.m.a. $1{\cdot}35 \pm 0{\cdot}2 \times 10^3$). Schmidt degradation of the acetic acid gave carbon dioxide devoid of activity.

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University Chemical Laboratories, Cambridge. [*Received, November 6th,* 1961.]

[14] Birch, *J.*, 1945, 809.