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trans-to-cis 2,3-DOUBLE BOND ISOMERIZATION OF EPOXYFARNESOL AND FARNESOL BY FUNGUS

Yoshikatsu Suzuki and Shingo Marumo

Department of Agricultural Chemistry, Nagoya University, Nagoya, Japan

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Naturally occurring farnesol has frequently been obtained as a mixture of its trans,trans- and cis,trans-isomers[1] accompanied by nerolidol. It has been established by Cornforth et al[2] that trans,trans-farnesol is built up biosynthetically from mevalonic acid through geraniol, and that the trans-double bond formation proceeds via elimination of the hydrogen of (4S)-mevalonic acid and not of (4R). The pathway by which cis,trans-farnesol is biosynthesized still remains ambiguous; whether direct biosynthesis from mevalonic acid occurs, or whether it is derived from trans,trans-farnesol by 2,3-double bond isomerization. However, it is generally accepted that the latter case may actually occur via nerolidol by an anionotropic rearrangement[3]. During the course of our investigation on the transformation of trans,trans-epoxyfarnesol (1) by Helminthosporium sativum, we found that the fungus partly converted 1 into the cis,trans-isomer (2b), and that dihydroxyfarnesol (4a), one of the metabolic products, was isolated from this fungus with a fair amount of the cis,trans-isomer (4b)*. This suggested that the trans-2,3-double bond might be isomerized to cis during the fungal transformation. We wish now to communicate that the trans-to-cis double bond isomerization of 1 by the fungus proceeds via the corresponding aldehydic intermediate (3), and also that trans,trans-farnesol (6) isomerizes by the same mechanism.

[1-D_2]-trans,trans-(±)-Epoxyfarnesol (1) was prepared by oxidation (active MnO_2 in acetone →active MnO_2-NaCN-AcOH-MeOH[4]) of [1-H_2]-1 followed by reduction with $LiAlD_4$. Complete replacement of the hydrogen atoms at C_1 by deuterium was confirmed by the nmr spectrum. The substrate (1, 180 mg) was added to the washed mycellium in a modified Czapeck-Dox medium (200 ml) as in the previous experiment[5]. After 11 hr, unchanged epoxyfarnesol (2, 50 mg) and dihydroxyfarnesol (4, 74 mg) were isolated from the culture filtrate. 2 was isolated and purified as acetate. In the nmr spectrum, a C_1-methylene proton had newly appeared at δ ca. 4.55 ppm.

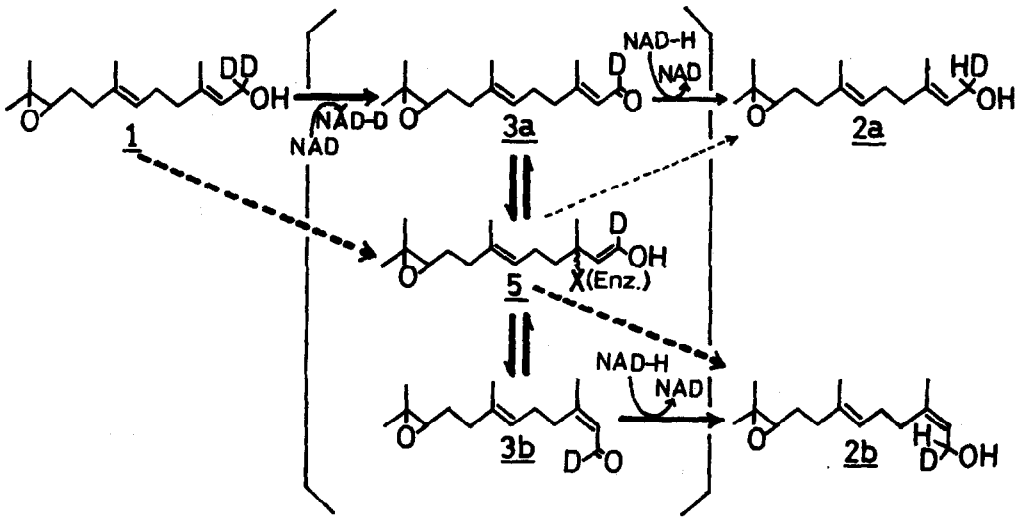
The overlapping pattern of this signal clearly indicated that the compound (2) isolated might be a mixture of trans,trans-, and cis,trans-isomers. The newly incorporated hydrogen to 2 was calculated as 0.365 atom %. A ratio of 21 % of the cis,trans- (2b) to 79 % of the trans,trans-isomer (2a) was determined from integration of the C_3-methyl signals of the cis- (δ1.73 ppm[6]) and the trans-isomers (δ1.66 ppm). 2a was purified by preparative thin-layer chromatography on silicic acid-$AgNO_3$, and the hydrogen incorporated in 2a was determined as 0.25 atom % from integration of the C_1-methylene proton signal. From these data, the exchange ratio of deuterium to hydrogen in 2b was calculated as 42 %. Thus, 0.84 atom % of the deuterium at the C_1-methylene position was replaced on isomerization of the double bond from trans to cis, and 0.25 atom % of the hydrogen was incorporated in 2a. Dihydroxyfarnesol (4) isolated from the culture filtrate was similarly analyzed and shown to be a mixture of the cis,trans- (4b) and trans,trans- (4a) isomers. The incorporated hydrogen was 0.66 in 4b and 0.21 atom % in 4a.

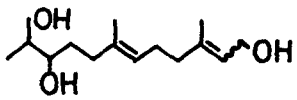
From these results, it can be deduced that the fungus dehydrogenated [D_2]-trans-allylic carbinol (1) enzymatically to [D_1]-trans-aldehyde (3a) or its biogenetically equivalent intermediate, which was then isomerized to the cis-aldehyde (3b), then reduced to the [D_1]-cis-carbinol (2b). As neither 3a nor 3b could be detected in the culture filtrate, these three reaction steps would seem to proceed synchronously. However, the alternative one-step reaction via the transition state (5) is not denied. As biological dehydrogenation of the carbinol to the aldehyde, and the reverse reduction are known to be catalyzed by dehydrogenase coupled with NAD(P) or its reductive form[7], the fact that complete deuterium replacement by hydrogen does not occur should be reasonably explained by assuming that the pool of NAD(P) in our experiment is not large enough for the NAD(P)-D produced on dehydrogenation of the substrate to be again used for its reduction.

Further evidence that the aldehyde (3) is a possible intermediate in the isomerization reaction was obtained by an experiment in which trans,trans-epoxyfarnesal (3a) was similarly metabolized by the washed mycellium (7 hr), affording** a mixture of 2a and 2b in the ratio of 68.6 to 31.4 % (54.8 % yield).

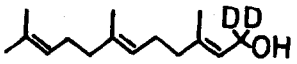
Next, we applied the same metabolic experiment to the more basal isoprenoid, trans,trans-farnesol. [1-D_2]-trans,trans-Farnesol (6, 85 mg) was incubated similarly. After 22 hr the farnesol (7) still remaining (37.4 mg) was isolated from the mycellium and found to be the

mixture of trans,trans- (7a, 92 %) and cis,trans- (7b, 8 %) isomers by glc analysis. The nmr analysis revealed that 7b contained 0.92 atom % of hydrogen, and 7a, 0.13 atom %. Thus, it was shown that the same type of isomerization reaction occurred on farnesol. In a preliminary experiment, geraniol was also shown to be isomerized to nerol.

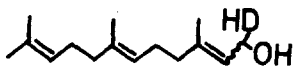




4a, trans
4b, cis



6



7a, trans
7b, cis

As the enzymatic isomerization of cis-trans double bonds in allylic alcohols has not previously been reported, this is the first finding on the new type of enzyme complex acting as a prenyl allylalcohol isomerase. The isomerization of double bonds conjugated with carbonyl groups has been shown to occur on maleate[8], maleylacetoacetate[9], maleylpyruvate[10], etc, and it may be said that these isomerizations and the isomerization of prenyl allylic alcohol here reported should proceed essentially by similar mechanisms. The present study also provides a useful method for defferentiating cis,trans-farnesol from the trans,trans-isomer as a biosynthe-

tic precursor of sesquiterpenoids. By the aid of this new method, a study on the biosynthesis of helminthosporol, a metabolite of this fungus, is now in progress to determine whether it derives from cis,trans-[11] or trans,trans-farnesol[12].

* S-(-)-10,11-Dihydroxyfarnesic acid, one of the metabolic products, was obtained as the completely pure trans,trans-form. trans,trans-Dihydroxyfarnesol, which was used for chemical derivation in our previous report, was therefore prepared from the ester by reduction with $LiAlH_4$.

** Dihydroxyfarnesol (4) was also obtained (9.2 mg). 4 was shown by the nmr spectrum to contain 33.8 % of the cis-isomer.

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