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STEREOCHEMISTRY OF C-1 HYDROGEN EXCHANGE DURING $\Delta^{2,3}$ TRANS-CIS ISOMERIZATION OF FARNESOL BY HELMINTHOSPORIUM SATIVUM

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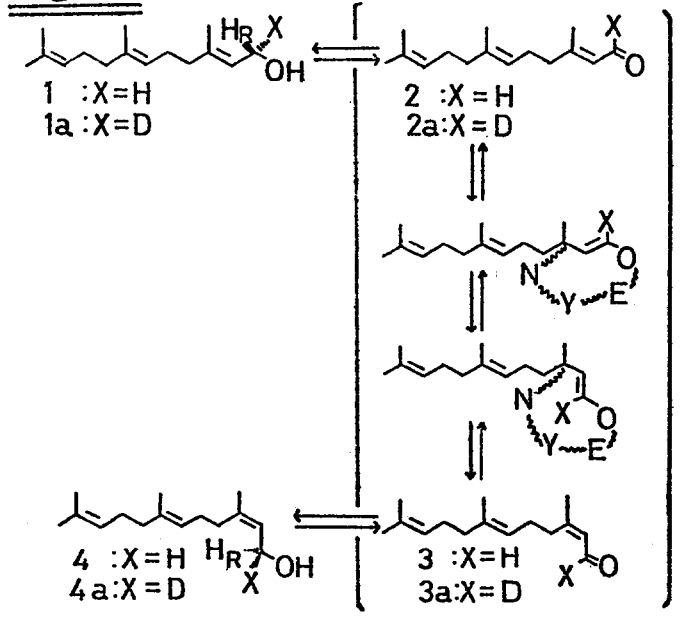
Farnesol is a common isoprenol encountered frequently in natural sources. The biosynthetic pathway to t,t-farnesol from mevalonic acid through geraniol has been established by Cornforth et al.[1] However, the one by which c,t-farnesol is derived has long been obscure. Recently, we have found that a fungus, Helminthosporium sativum, catalyzes trans-to-cis isomerization of farnesol when the t,t isomer is added to the culture medium, and this isomerization was shown to proceed via an aldehydic intermediate, farnesal, with loss of one hydrogen at the C-1 methylene of the t,t substrate.[2] Since that time, several publications[3] which described a similar pathway to operate in certain kinds of plants and fungi appeared. This report describes the stereochemistry of the hydrogen exchange in the courses of dehydrogenation and hydrogenation, taking an important part in this isomerization by the fungus.

Two enzymatic processes must be stereochemically clarified, i.e., the hydrogen exchange in the reversible conversion between t,t-farnesol (1) and t,t-farnesal (2), and between c,t-farnesal (3) and c,t-farnesol (4). The former process was investigated by the fungal transformation of [1-D]-t,t-farnesal (2a) as a substrate, which was prepared from methyl t,t-farnesoate by reduction ($LiAl^2H_4$) followed by oxidation (MnO_2). The substrate was shaken with precultured mycelia of H. sativum in a modified Czapek-Dox medium for 8 hr in the same way as in the previous work.[2] Chromatographic purification of the ethyl acetate extracts of the culture filtrate gave two hydrogenation products: [1-DH]-t,t-farnesol (1a), $[\alpha]_D^{28}$ $+0.69^\circ \pm 0.09^\circ$ (c, 1.4 in MeOH) (58.7%), the [1-DH]-c,t-isomer (4a) (9.6%), $[\alpha]_D^{25}$ $+0.34^\circ$ (c, 4.1 in MeOH). For comparison of the former optical rotation, authentic S(+)-[1-DH]-t,t-farnesol was synthesized according to the method of Streitwieser Jr. et al,[4] showing $[\alpha]_D^{28}$ $+0.24^\circ \pm 0.15^\circ$ (c, 2.4 in MeOH). The fact that the fungal t,t product had a positive optical rotation strongly suggested 1a to have the S configuration. The suggestion was unequivocally confirmed by dehydrogenation of 1a with horse liver

alcohol dehydrogenase (HL-ADH),[5] since the enzyme is known to abstract the pro-R hydrogen from the appropriate methylene of isoprenols such as geraniol.[6] t,t-Farnesal obtained by the enzymatic dehydrogenation of 1a was converted into a 2,4-dinitrophenylhydrazone (2,4-DNP) derivative, and the deuterium content was determined by a mass spectrometric analysis. From the peak intensities at m/e 401 and 400, which were assigned to the respective M^+ ions of [1-D]- and [1-H]-farnesal-2,4-DNP, it was calculated that ca. 95% of the deuterium in 1a was retained in the product. The ca. 5% incorporation of hydrogen into the 2,4-DNP derivative should be attributed to contamination during the hydrazone-formation, because even isotopically pure [1-D]-farnesal was contaminated with ca. 5% hydrogen after the hydrazone-formation. Thus, the hydrogenation process, 2a ⟶ 1a, by the fungus has been clarified to proceed, with almost complete stereospecificity, in such a stereochemical manner that the hydrogen was introduced onto the aldehydic group of 2a from the re-face, as shown in the Figure, forming S(+)-[1-DH]-t,t-farnesol.

Whether such a stereospecific exchange of C-1 hydrogen occurs in the reverse reaction, 1a ⟶ 2a, was examined by feeding synthetic S(+)-[1-DH]-t,t-farnesol (D content 100% from nmr spectrum; optical purity 38% from the HL-ADH (NAD^+) reaction) to the fungus. The c,t-farnesal produced

Figure



Y: an enzyme, or other substance. N: nucleophilic center. E: electrophilic center.

(5.8%) was converted into its acetate. Mass spectrometric analysis of the acetate revealed that 67.4% of the deuterium originally present in the substrate was retained in the isomerized product; this percentage is in fairly good agreement with the 69% calculated from the optical purity of the synthetic farnesol. This showed the pro-R hydrogen to be abstracted in this process. Thus, the hydrogen exchanged during the reversible dehydrogenation-hydrogenation process, 1 ⟷ 2, has been shown to be pro-R.

Next, the hydrogenation of c,t-farnesal, 3 ⟶ 4, was investigated. As the stereochemical aspect of HL-ADH (NAD^+) dehydrogenation on c,t-farnesol had not been clarified yet, synthetic S-[1-DH]-c,t-farnesol (D content 100% from nmr spectrum) was exposed to this enzymatic reaction. Deuterium content in the product, c,t-farnesal, was mass-spectrometrically determined, after converting into the 2,4-DNP derivative to be 66% of the original. This showed that the enzyme also abstracts the pro-R hydrogen from the c,t substrate. Then, [1-D]-c,t-farnesal (3a) was subjected to the fungal transformation under the same condition as in the case of t,t-farnesal, and the resulting two hydrogenation products, [1-DH]-c,t-farnesol (4a) (53.7%) and the t,t isomer (1a) (10.8%), were isolated in pure form. A product 4a (D content 100%) was exposed to HL-ADH (NAD^+) dehydrogenation, and the deuterium content in the product, c,t-farnesal, was analyzed by mass-spectrometry, with the result that 100% deuterium (compensated for 5% contamination) of the substrate was retained in the product. These results unequivocally indicated that the process, 3a ⟶ 4a, proceeds, with almost complete stereospecificity, by introducing a hydrogen onto the aldehydic group of 3a from the re-face, producing S(+)-[1-DH]-c,t-farnesol. The result also showed that HL-ADH dehydrogenation of c,t-farnesol proceeds with almost complete stereospecificity.

The over-all processes of fungal trans-cis isomerization of farnesol have been thus clarified as shown in the Figure, in which an alcohol dehydrogenase plays an important role, exchanging the pro-R hydrogen stereospecifically. Although the mechanism of isomerization of farnesal has not been clarified yet, we speculate that the process may include intermediary enolic forms, presumably bound with an enzyme (or, other substances having both nucleophilic and electrophilic centers). An interesting remaining problem is whether this isomerization reaction is catalyzed by a particular enzyme, or proceeds non-enzymatically with the aid of a proteinaceous substance.

Quite recently, Overton and Roberts[7] reported on a similar study using tissue culture of Andrographis paniculata. They showed that while the pro-R hydrogen was abstracted in the cis-to-trans process, the pro-S hydrogen was eliminated in the reverse trans-to-cis reaction. Thus, there is an interesting difference between the results with plant and fungus.

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