Short Communication

Formation of (2*S*,6*R*,7*R*)-2,7-Epoxydihydro- α -ionone via Asymmetric Hydroxylation of β -Ionone by *Aspergillus niger*

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Received September 24, 1980

In the previous paper, the usefulness of microbial conversion in the preparation of naturally occurring aroma compounds derived from the parent terpenoids such as carotenoid has been demonstrated. β -Ionone was converted into more than 13 compounds containing (*S*)-2-hydroxy- β -ionone as a major product by a selected strain of *Aspergillus niger*.^{1,2)} In the present paper, we wish to report the identification and synthesis of a chiral oxabridged ionone, (2*S*, 6*R*, 7*R*)-2,7-epoxydihydro- α -ionone, which was formed from β -ionone as a minor product ($\sim 0.9\%$) in the culture broth of the fungus on prolonged incubation at rather high pH.

The spores (4×10^7) of *Aspergillus niger* JTS 191 were inoculated and cultivated in a liter of a medium consisting of 3% of sucrose, 0.2% NaNO₃, 0.1% K₂HPO₄, 0.05% KCl, 0.05% MgSO₄ · 7H₂O, 0.1% yeast extract and distilled water for 48 hr at 28°C under rotary shaking. To the resulting culture broth, 0.1% (wt/vol) of β -ionone was added as a substrate. The broth was incubated at 28°C under continuous shaking. After 14 day's incubation without adjustment of pH, the culture broth was extracted with ethyl acetate and concentrated *in vacuo*. Silicic acid column chromatography and preparative gas chromatography gave a pure compound **1**. $[\alpha]_{546}^{23} + 21.6^\circ$ ($c=0.2$, methanol), MS m/z : 208 (M^+ , 2), 150 (2), 135 (1), 122 (11), 107 (100), 93 (30), 91 (10), 79

(7), 77 (6), 55 (4), 53 (4), 43 (26), 41 (8). PMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.08 (s, 3H, CH₃-C(1)), 1.20 (s, 3H, CH₃-C(1)), 1.60 (s, 3H, CH₃-C(5)), 1.95 (d, 1H, $J_{6,7}=4$ Hz, H-C(6)), 2.17 (s, 3H, CH₃-C(10)), 2.70 (2H, center of AB part in H_xC(7)-H_AH_BC(8), $J_{A,B}=16$ Hz, $J_{A,X}=7$ Hz, H_A-C(8) (2.54) and H_B-C(8) (2.84): H₂-C(8)), 3.64 (m, 1H, H-C(2)), 4.66 (dxdxd, 1H, H-C(7)), 5.33 (m, 1H, H-C(4)). IR $\nu_{max}^{film} \text{ cm}^{-1}$: 1718, 1219, 1162, 1049, 1008, 1002, 954, 942, 926, 915, 865, 848.

On the basis of the data presented above, **1** was assigned the 2,7-epoxydihydro- α -ionone structure. This compound has been prepared as an intermediate in the synthesis of oxabridged megastigmadienes.^{3,4)} The spectral data of the isolated **1** were identical with those of the synthetic compound except the optical activity.

Although 2,7-epoxydihydro- α -ionone has three asymmetric carbons in its molecule, 4 isomers are theoretically possible, because only 2,6-*cis* configuration could give the oxabridge. The optical purity of isolated **1** was examined by PMR using a chiral shift reagent, according to the method of Goering *et al.*⁵⁾ The shifted spectra of **1** showed the absence of other diastereomeric adducts. The integrity of **1** was also confirmed by GLC equipped with glass capillary columns (PEG 20M and Silicon DC SE-30).

The synthetic route reported for 2,7-epoxydihydro- α -ionone³⁾ and isomerization of irone,⁶⁾ together with the evidence that the higher pH of the culture broth accelerated the formation of **1**, suggested that **1** would be formed from (*S*)-2-hydroxy- β -ionone by oxabridged ring closure *via* base catalyzed isomerization in the culture broth.

Then, (*S*)-2-hydroxy- β -ionone was dissolved in absolute ethanol containing 5% of sodium ethoxide, and refluxed at 45°C for 4 hr. The optically active 2,7-epoxydihydro- α -ionone was obtained at 35% yield after purification by preparative HPLC; $[\alpha]_{546}^{23} + 20.6^\circ$ ($c=0.3$, methanol). The PMR spectra were identical with those of **1**.

Judging from the configuration of the

starting material, the synthesized 2,7-epoxydihydro- α -ionone should have the (2*S*, 6*R*)-configuration. The Dreiding model inspection shows that in (2*S*, 6*R*, 7*R*)-configuration, the dihedral angle of H-C(6)-C(7)-H is about 30° , whereas in (2*S*, 6*R*, 7*S*)-configuration, the angle is about 78° . According to Karplus equation,⁷⁾ the vicinal coupling constant, $J_{6,7}$, of the former is calculated at 6.1 Hz, and that of the latter at 0 Hz. Therefore, the measured coupling constant of **1** and the synthesized one, $J_{6,7}=4$, cogently supports the (7*R*)-configuration.

In conclusion, the absolute configuration of **1** and the synthesized compound was deduced to be (2*S*, 6*R*, 7*R*).

Though the ring closure is the Michael type addition, the chiral compound **1** was produced under the synthetic conditions as well. The optical purity measured by PMR with $\text{Eu}(\text{TFC})_3$ ⁵⁾ depended on the reaction tem-

perature; at 60°C , 7*R*:7*S*=80:20, and at 45°C , 7*R*:7*S*=100~95:0~5. Judging from the Dreiding model inspection, this would be due to the fact that (2*R*, 6*R*, 7*S*)-configuration is sterically more hindered than the diastereomeric (2*S*, 6*R*, 7*R*)-counterpart. At 40°C , the ring closure was not effected under the synthetic conditions. The experiments failed in the formation of **1** from (*S*)-2-hydroxy- β -ionone in the alkaline culture media without the mycelial pellets at 28°C . These results suggest that the formation of **1** from (*S*)-2-hydroxy- β -ionone would be characteristic of microbial process.

Recently, several oxabridged ionones were found as aroma constituents of tobacco⁸⁾ and *Osmanthus*.⁴⁾ **1** had a floral note and was useful for tobacco flavoring on ppm level.

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