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STEREOSELECTIVE EPOXIDATION OF GERMACRONE BY *CUNNINGHAMELLA BLAKESLEEANA*

H. Hikino, C. Konno, T. Nagashima, T. Kohama, and T. Takemoto

Pharmaceutical Institute, Tohoku University, Sendai, Japan.

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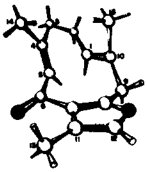
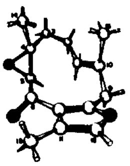
Germacra-1(10),4-diene derivatives are thought to be precursors of a number of bicarbocyclic sesquiterpenoids. It is considered that the transformation from the former to the latter is initiated mainly by epoxidation of a double bond followed by cyclization of the resultant epoxide, during which the first epoxidation process is stereospecific since it must be conducted by an enzyme. In fact, we[1,2] have previously shown that furanodienone and zederone, constituents of *Curcuma zedoaria* Roscoe (Zingiberaceae), possess the absolute configurations and conformations as shown in stereoformulas I* and II, respectively, demonstrating that the oxidation enzyme of the plant attacks stereospecifically on one (I) of the two enantiomeric conformers of furanodienone to afford zederone (II). Our interests on the stereospecificity of the reaction caused us to examine the enzymatic epoxidation of germacrone (III) which is another constituent of *C. zedoaria* and is considered to be a precursor of a variety of bicarbocyclic sesquiterpenoids in this plant. The present fermentation study has revealed a bio-oxygenative transformation, the stereoselective epoxidation by a microbe of isolated unsaturation in terpenoids having no asymmetric centers.


I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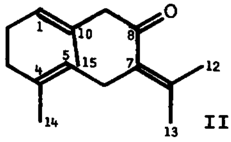
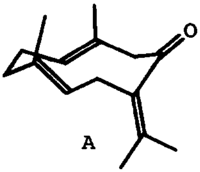


III

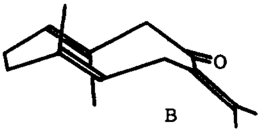
It has been known in the steroid field that epoxidation occurs when substrates with isolated double bonds are incubated with several microbes.[3] We therefore studied these microorganisms in the hope that stereoselective epoxidation of germacrone by enzymes induced in the microbes could be achieved as it is in the plant. From the initial screening results, it was found that *Cunninghamella blakesleeana* is capable of transforming germacrone in fairly high efficiency.

On incubation with the microbe, germacrone gave three main products (IV, V, VI) which were separated by silica gel chromatography. The least polar product (IV), $C_{15}H_{22}O_2$, mp 63-64°, the second product (V), $C_{15}H_{22}O_2$, mp 80.5-81.5°, and the third product (VI), $C_{15}H_{22}O_3$, mp 123.5-124.5°, were all shown to retain the 7(11)-en-8-one chromophore from their spectral properties (252 nm, 1680 cm^{-1}, 1.66, 1.76 ppm; 240 nm, 1680 cm^{-1}, 1.81, 1.81 ppm; and 250 nm, 1680 cm^{-1}, 1.78, 1.85 ppm, respectively) and have no hydroxyl groups. In the NMR spectrum of germacrone, the C-1 and C-15 proton signals and the C-5 and C-14 proton signals occurred at 4.97 and 1.62 ppm, and 4.72 and 1.45 ppm, while the spectrum of the second product (V) showed the corresponding signals at 5.20 and 1.71 ppm, and 2.42 and 1.03 ppm, the latter pair suffering from higher field shifts. Analysis of the spectrum revealed that the vinyl proton signal at 5.20 ppm constitutes an ABX spectrum along with the methylene proton signals at 2.74 and 3.10 ppm. These findings established the product (V) to be germacrone 4,5-epoxide. On the other hand, the spectrum of the first product (IV) displayed the corresponding signals at 2.53 and 1.17 ppm, and 4.96 and 1.50 ppm, the former pair being shifted towards higher fields. This observation demonstrated that the product (IV) is germacrone 1,10-epoxide. Further, the spectrum of the third product (VI) disclosed the methyl proton signals at higher fields (1.14 and 1.43 ppm) and showed no vinyl proton signal, indicating it to be germacrone 1,10;4,5-diepoxide. In confirmation, epoxidation of germacrone with perbenzoic acid was carried out to give two monoepoxides and a diepoxide which were found identical with the epoxides (IV, V, VI) except for the optical rotations.

The next problem was to determine the conformations of the epoxides (IV, V, VI). Then, the monoepoxides (IV, V) were oxidized with perbenzoic acid yielding diepoxides which were identified as the diepoxide (VI) but exclusive of optical rotations. Since in these epoxidation reactions an oxygen atom must be added to an ethylene bond from the outside of the ten-membered ring and since a conformational change, which makes the epoxide ring locate inside of the ten-membered ring, is thought to be highly unlikely due to the steric hindrance, all the epoxides (IV, V, VI) are concluded to adopt similar conformations. In the NMR spectrum of the epoxide (V), an intramolecular nuclear Overhauser effect was observed between the C-1 hydrogen and the C-5 hydrogen, indicating that both hydrogens reside in the *syn* relationship. Since the C-1:C-10 and C-4:C-5 ethylenic linkages in germacrone are both *trans*,[4)] the C-4 and C-10 methyl groups are also *syn*. In the NMR spectra of the epoxides (IV, V), the signals due to the hydrogen and the methyl in a trisubstituted double bond appear in lower-fields than the corresponding signals in the spectrum of germacrone. These displacements in the chemical shifts which are interpreted by the difference in anisotropic shielding effects of a double bond and an epoxide ring, demonstrated that the planes which involve the C-1:C-10 and C-4:C-5 double bonds are situated in a parallel relationship.2) The solvent-induced shifts for the C-12 and C-13 methyl proton resonance (δ_{CDCl_3}-$\delta_{C_6D_6}$) in germacrone (III) and its epoxides (IV, V, VI) show large positive values (+0.30 to +0.50 ppm), a fact which cannot be explained by the *s-cis* but the *s-trans* arrangement of the enone system.5) Combined evidence has thus determined that germacrone (III) and its epoxides (IV, V, VI) have conformations similar to A*. Since the conformation A for germacrone was in-



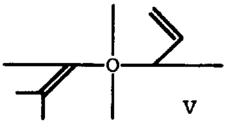
A



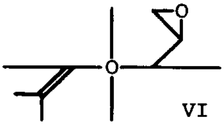
B

consistent with the conformation B postulated by Sorm,6) the silver nitrate adduct of germacrone was subjected to X-ray crystal structure analysis.† The result pointed to the conformation A for germacrone, which is in support of our conclusion.

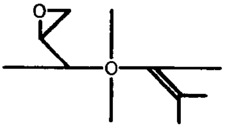
The remaining problem was the absolute configurations of the epoxides (IV, V, VI). The diepoxide (VI) obtained from germacrone by fermentation and that yielded from the epoxide (V) by perbenzoic acid oxidation gave the similar ORD curves showing positive Cotton effects for the *n*-π* transition, indicating that both epoxides (V, VI) possess the same absolute configuration. While the ORD curve of the epoxide (V) itself exhibits a negative *n*-π* Cotton effect. These observations indicate that the α,β-unsaturated carbonyl group in the epoxides (V, VI) has the chirality which shows a positive Cotton effect and, furthermore, the β,γ-unsaturated carbonyl group in the epoxide (V) lies in the relation so as to give a negative contribution.7) On the



V

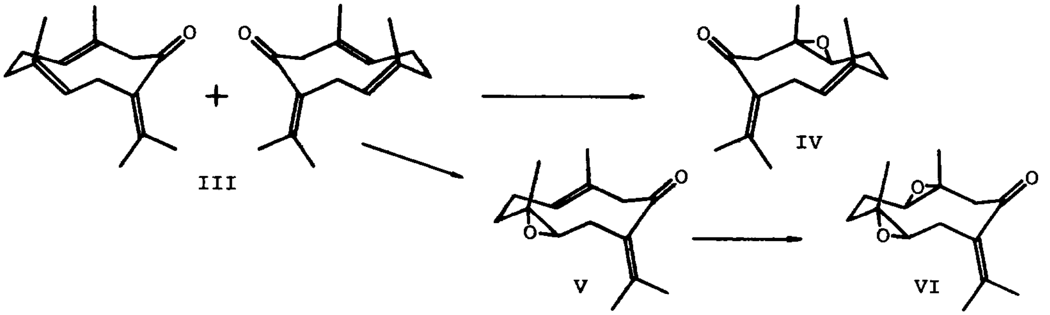


VI



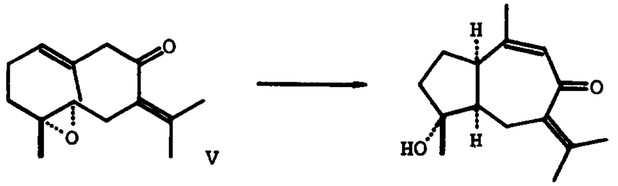
IV

other hand, the diepoxide formed from the epoxide (IV) by perbenzoic acid oxidation gave the ORD curve exhibiting a negative *n*-π* Cotton effect, demonstrating that the epoxide (IV) has the oppo-



site absolute configuration to the epoxides (V, VI). In accord with this deduction, the ORD curve of the epoxide (IV) itself shows a negative n-π^* Cotton effect as is expected from the octant diagram.7) The epoxides (IV, V, VI) are thus concluded to have the stereostructures above.

Since the amplitudes of the ORD curves of the diepoxides from different sources were not constant, the optical purities of the epoxides (IV, V, VI) were thought to be doubtful. Then the epoxide (V) was subjected to rearrangement under various conditions. As the results, it was found that treatment of the epoxide (V) ($[\Phi]_{330}$ -680, $[\Phi]_{276}$ +410) with *p*-toluenesulphonic acid in benzene at -20° furnished procurcumenol8)‡ ($[\alpha]_D$ +36°). Comparison of this optical rotation with that of the natural procurcumenol (VII) ($[\alpha]_D$ +141°) revealed that although the enzymatic epoxidation of germacrone proceeds stereoselectively, the specificity is not complete.


V VII

It is quite interesting that the enantiomeric conformation of either germacrone (III)8) or furanodienone (I)2,9) which is attacked preferentially during enzymatic oxidation, is the same for both the microorganism and the plant.

We are indebted to Dr. K. Kuriyama, Shionogi Research Laboratory, for the ORD data, and to Research Laboratories, Takeda Chemical Industries, Ltd., and Research Laboratories, Sankyo & Co., Ltd., for the NMR spectra.

FOOTNOTES AND REFERENCES

* including its enantiomeric conformation.

† The X-ray crystallographic study was performed by Dr. M. Nishikawa, Research Laboratories, Takeda Chemical Industries, Ltd., to whom our sincere thanks are due.

‡ In connection with this result, procurcumenol is established to have the stereostructure VII.

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