[Chem. Pharm. Bull. 25(1) 6—18 (1977)]

UDC 547.913.04 : 542.98

Stereoselective Epoxidation of Germacradienes by Enzymes and Selective Transannular Cyclization of Germacradienes and Their Epoxides[1]

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(Received May 30, 1975)

Microbial transformation of germacrone (**5**) using *Cunninghamella blakesleeana* has resulted in the stereoselective attack of oxidation enzymes to give three optically active epoxides (**6**, **7**, and **8**) whose stereostructures have been elucidated by chemical and physicochemical evidence. Transannular cyclization of germacrone 1,10-epoxide (**6**) and germacrone 4,5-epoxide (**7**) by acids has been performed and it has been found that the former gives the eudesmanes (**10** and **11**) while the latter affords the guaianes (**9** and **12—15**). The mechanism of acid-induced transannular rearrangement of germacra-1(10), 4-dienes and their epoxides is discussed.

Keywords——*Cunninghamella blakesleeana*; enzyme reaction; germacradiene; microbiological transformation; stereoselective epoxidation; transannular cyclization

We have been working on the constituents of zedoary, the rhizomes of *Curcuma zedoaria* Roscoe belonging to Zingiberaceae, from which a number of sesquiterpenoids have been isolated.[3] Among these isoprenoids, there are several sesquiterpenoids of the germacra-1(10),4-diene type such as germacrone, furanodiene,[4] and furanodienone.[5]

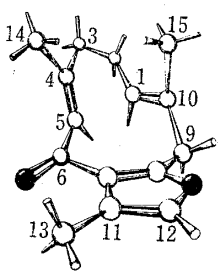
Biosynthetically, sesquiterpenoids of the germacradiene type are thought to be precursors of a number of bicarbocyclic congeners. It is considered that the transformation from the former to the latter is effected by attack of acid to an ethylene bond followed by transannular cyclization or by epoxidation of a double bond followed by cleavage of the resultant epoxide ring concomitant with transannular cyclization.

Among possible intermediates, monoepoxides of the germacradiene derivatives, in the biogenesis of sesquiterpenoids in zedoary, zederone has actually been isolated.[6,7]

Since the germacra-1(10),4-diene moiety itself has no asymmetric center, the germacradiene derivatives show no optical activities unless they have asymmetric carbons in the other parts of the molecules. However, monoepoxides of germacradienes and their rearranged products in nature exhibit optical activities. Therefore, the initial epoxidation of the germacradiene intermediates must be stereoselective. This is quite probable because this process should be conducted by plant enzymes. We are interested in the stereoselectivity of the biological epoxidation process of the germacradiene derivatives.[8]

The first example is the biogenesis of zederone (**2**). Although its exact mode of biosynthesis is not known, zederone (**2**) is most probably derived from furanodienone (**1**) through

1) Part XII in the series on Biochemical Synthesis. This paper also constitutes Part L in the series on Sesquiterpenoids.
2) Location: *Aoba-yama, Sendai.*
3) H. Hikino, C. Konno, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 987 (1972).
4) H. Hikino, K. Agatsuma, C. Konno, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 752 (1970).
5) H. Hikino, C. Konno, K. Agatsuma, T. Takemoto, I. Horibe, K. Tori, M. Ueyama, and K. Takeda, *J. Chem. Soc. Perkin I*, **1975**, 478.
6) H. Hikino, S. Takahashi, Y. Sakurai, T. Takemoto, and N.S. Bhacca, *Chem. Pharm. Bull.* (Tokyo), **16**, 1081 (1968).
7) H. Hikino, K. Tori, I. Horibe, and K. Kuriyama, *J. Chem. Soc.* (*C*), **1971**, 688.
8) Part of the material descrived herein has been previously reported in a preliminary form: H. Hikino, C. Konno, T. Nagashima, T. Kohama, and T. Takemoto, *Tetrahedron Letters*, **1971**, 337.



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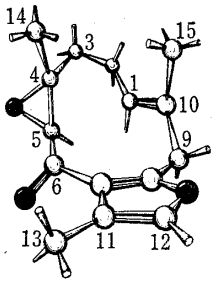

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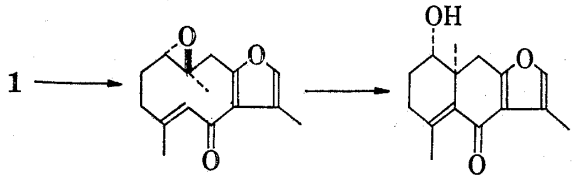


4 3

stereoselective epoxidation of the C-4: C-5 double bond by an enzyme. The second example is the biogenesis of curcolone (**3**) which is known as another constituent of the same plant, *C. zedoaria*.[9] It is considered that this terpenoid is derived from the intermediate (**4**) which can be formed through stereoselective epoxidation of the C-1: C-10 double bond of furanodienone (**1**). Now it is evident that oxidation enzymes in the plant attack stereoselectively on the double bonds in the ten-membered ring of furanodienone (**1**).

Our interests on the stereoselectivity of the epoxidation reaction caused us to examine the microbiological epoxidation of germacrone (**5**) which is another constituent of the same plant and is considered to be a precursor of a variety of bicarbocyclic sesquiterpenoids in this plant. There was, however, no inevitability that microbial transformation of germacrone would give its epoxides. Since it has been known in the steroid field that epoxidation occurrs when substrates with isolated double bonds are incubated with several microbes such as *Cunninghamella blakesleeana*, *Curvularia lunata*, *Helicostylum piriforme*, and *Mucor griseocyanus*,[10] we studied these microorganisms in the hope that stereoselective epoxidation of germacrone by enzymes induced in the microbes could be achieved. From initial screening results, it was found that some microbe did not metabolize germacrone very much, some afforded a number of polar products having hydroxyl groups, and *Cunninghamella blakesleeana* was capable of transforming germacrone in fairly high efficiency.

On incubation with *C. blakesleeana*, germacrone gave three main products (**6**, **7**, and **8**) which were separated by silica gel chromatography. The least polar product (**6**) and the second product (**7**) possess the same composition $C_{15}H_{22}O_2$ one oxygen atom more than the substrate, germacrone, and the third product (**8**) has the molecular formula $C_{15}H_{22}O_3$ two oxygen atoms more than the substrate. All these products were 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 also shown to have no hydroxyl groups. In the nuclear magnetic resonance (NMR) spectrum of germacrone, the C-1 and C-15 hydrogen signals and the C-5 and C-14 hydrogen signals occurred at 4.97 and 1.62 ppm, and 4.72 and 1.45 ppm, while the spectrum of the first product (**6**) showed the signals at 2.53 and 1.17 ppm, and 4.96 and 1.50 ppm, the former pair being shifted towards higher fields, a fact which suggested the product (**6**) to be either germacrone 1,10-epoxide or germacrone 4,5-epoxide. Nuclear magnetic double resonance (NMDR) experiments revealed that the vinyl hydrogen signal at 4.96 ppm constitutes an ABX spectrum along with the methylene hydrogen signals at 2.74 and 3.10 ppm.[11] These observations demonstrated that the product (**6**) is germacrone 1,10-epoxide. On the other hand, the spectrum of the second product (**7**) displayed the corresponding signals at 5.20 and 1.72 ppm, and 2.42 and 1.03 ppm, the latter pair suffering from higher field shifts. These findings

9) H. Hikino, Y. Sakurai, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 827 (1968).
10) H. Iizuka and A. Naito, "Microbial Transformation of Steroids and Alkaloids," Univ. of Tokyo Press, Tokyo, 1967.
11) This result was erroneously reported as that for the second product (**7**) in the preliminary communication.[8]

indicated that the 4: 5 double bond was oxidized to form the 4,5-epoxide (**7**) during the fermentation of germacrone. Saturation of the vinyl hydrogen signal at 5.20 ppm only brought about signal changes in the region 2.1—2.4 ppm which could not be well analyzed. Based on the above evidence, the product (**7**) was concluded to be germacrone 4,5-epoxide. Further, in the spectrum of the third product (**8**), the two vinyl methyl hydrogen signals and two vinyl hydrogen signals observed in the spectrum of germacrone disappeared, and instead two signals due to tertiary methyl hydrogens occurred at 1.14 and 1.43 ppm and two signals associated with hydrogens on carbons bearing oxygen functions appeared at 2.62 and 2.64 ppm, showing 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 one diepoxide which were found identical with the fermentation products (**6**, **7**, and **8**) except that the epoxides obtained by peracid oxidation are optically inactive while the frementation products are optically active.

In order to establish these epoxidations as *bona fide* enzymatic reactions and eliminate the possibility of air oxidation of substrate, control experiments were carried out under the same conditions except for the absence of the microbe. Under these conditions epoxidation of unsaturated substrate was not observed, thereby demonstrating that the process was enzymatic in nature.

The next problem was to determine the absolute configurations of the epoxides obtained by microbial transformation. For this purpose, the conformations of these epoxides must be first established, because the germacrane skeleton can adopt various conformations, and optical rotatory dispersion (ORD) and circular dichroism (CD) data, which should provide evidence for its absolute configuration, do not give information about the stereochemistry in the absence of knowledge of the molecular conformation. The two monoepoxides (**6** and **7**) obtained from fermentation were oxidized with perbenzoic acid to give diepoxides which were identified as the diepoxide (**8**) except for the optical rotations which will be discussed later. 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 located inside of the ten-membered ring, is thought to be unlikely due to the steric hindrance, all the epoxides (**6**, **7**, and **8**) are concluded to adopt similar conformations. In the NMR spectrum of the 4,5-epoxide (**7**), an intramolecular nuclear Overhauser effect (NOE) was observed between the C-1 and C-5 hydrogens, indicating that both hydrogens are *syn*. Since the 1,10- and 4,5-double bonds in germacrone are both E (*trans*),[12] the C-4 and C-10 methyl groups are also *syn*. In the NMR spectra of both the monoepoxides (**6** and **7**),

Table I. NMR Chemical Shifts

	H-1	H-5	H-12	H-13	H-14	H-15
Furanodienone (**1**)[a]	5.16	5.80	7.05	2.13	1.99	1.30
Zederone (**2**)[a]	5.47	3.78	7.07	2.10	1.33	1.60
Germacrone (**5**)[a]	4.97	4.72	1.72	1.77	1.45	1.62
Germacrone (**5**)[b]	4.75	4.75	1.42	1.46	1.36	1.61
Germacrone 1,10-epoxide (**6**)[a]	2.53	4.96	1.66	1.76	1.50	1.17
1,10-epoxide (**6**)[b]	2.50	4.87	1.31	1.41	1.37	1.27
Germacrone 4,5-epoxide (**7**)[a]	5.20	2.42	1.81	1.81	1.03	1.72
4,5-epoxide (**7**)[b]	4.87	2.35	1.36	1.39	0.94	1.61
Germacrone 1,10;4,5-diepoxide (**8**)[a]	2.64	2.62	1.78	1.85	1.14	1.43
1,10;4,5-diepoxide (**8**)[b]	2.64	2.43	1.33	1.35	1.01	1.28

a) in $CDCl_3$ b) in C_6D_6

12) F.H. Allen and D. Rogers, *Chem. Commun.*, **1967**, 588.

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 (Table I). 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 1,10- and 4,5-double bonds are situated nearly in a parallel relationship, as discussed in the conformations of furanodienone (1) and zederone (2).[7] The solvent-induced shifts for the C-12 and C-13 methyl hydrogen resonances ($\delta_{CDCl_3}-\delta_{C_6D_6}$) in germacrone and its three epoxides (**6**, **7**, and **8**) show large positive values (+0.30—+0.50 ppm) (Table I), a fact which cannot be explained by the *s-cis* but the *s-trans* arrangement of the enone system.[13] Combined evidence has thus determined that germacrone and its epoxides (**6**, **7**, and **8**) have conformations similar to A. However, this conformation A for germacrone was inconsistent with the conformation B postulated for germacrone by Šorm.[14] Therefore, the silver nitrate adduct of germacrone was subjected to X-ray crystal structure analysis whose results indicated the conformation for germacrone as shown in Fig. 1 which is in support of our conclusion.

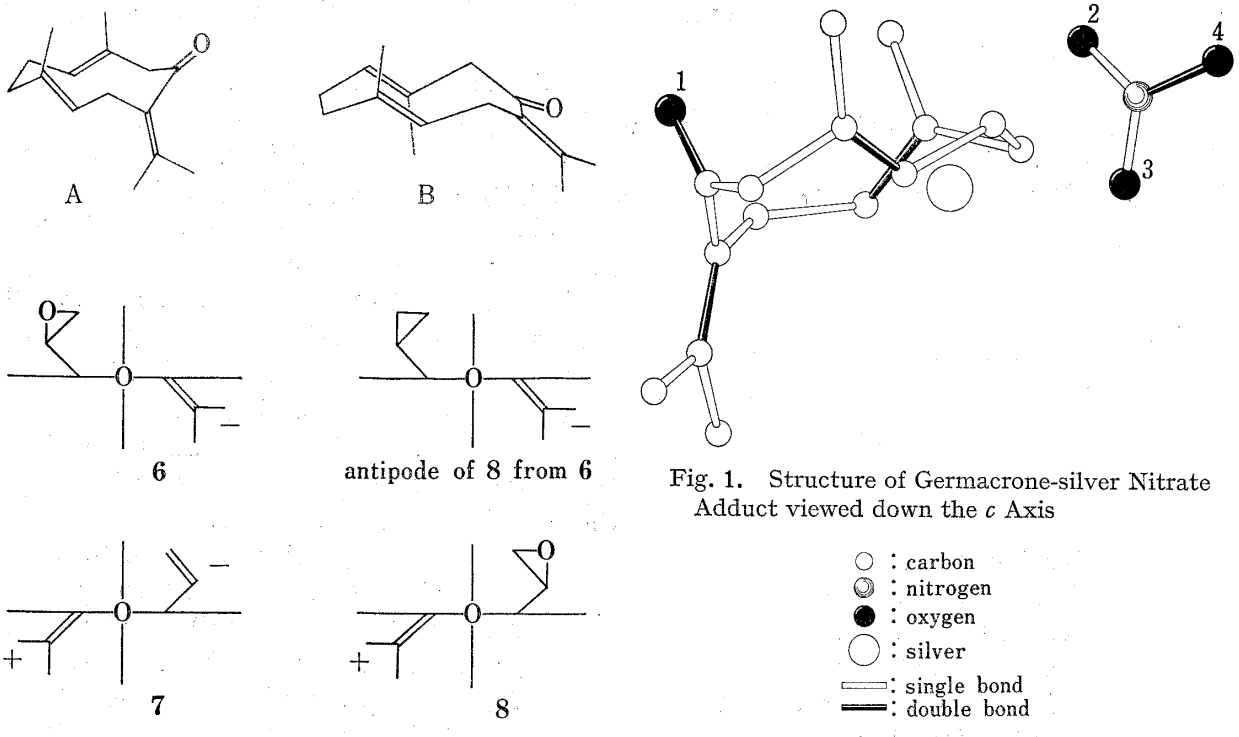
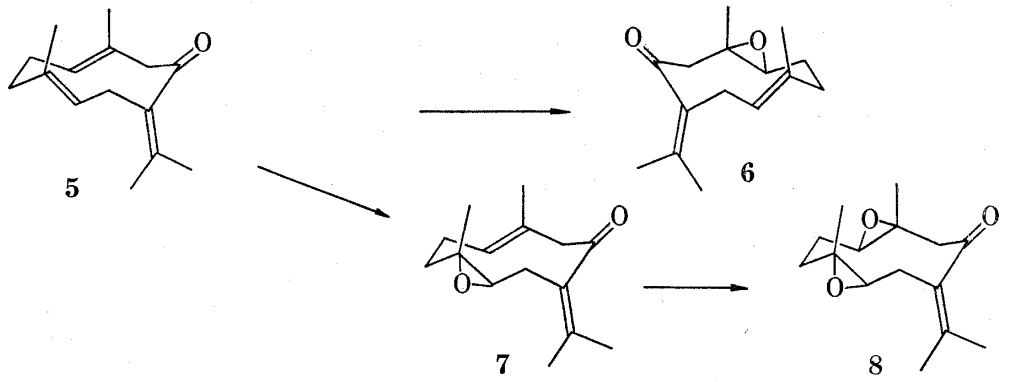


Fig. 1. Structure of Germacrone-silver Nitrate Adduct viewed down the *c* Axis

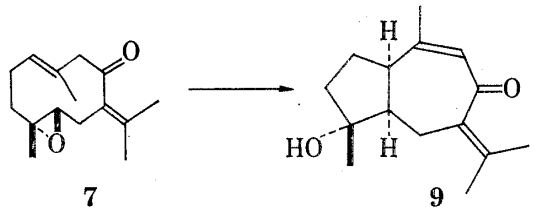
The remaining problem was the absolute configurations of the epoxides (**6**, **7**, and **8**). The diepoxide (**8**) obtained from germacrone by fermentation and the diepoxide yielded from the 4,5-epoxide (**7**) by perbenzoic acid oxidation gave the similar ORD curves showing positive Cotton effects for the n—π^* transition. While the ORD curve of the 4,5-epoxide (**7**) itself exhibits a negative n—π^* Cotton effect. These observations indicate that the α,β-unsaturated carbonyl system in the epoxides (**7** and **8**) has the chirality which shows a positive Cotton effect and, furthermore, the β,γ-unsaturated carbonyl system in the 4,5-epoxide (**7**) lies in the relation so as to give a negative contribution.[15] On the other hand, the diepoxide formed from the 1,10-epoxide (**6**) by perbenzoic acid oxidation gave the ORD curve exhibiting a negative n—π^* Cotton effect, demonstrating that the 1,10-epoxide (**6**) has the opposite absolute configuration to the other epoxides (**7** and **8**). In accord with this deduction, the ORD curve

13) C.J. Timmons, *Chem. Commun.*, **1965**, 576.
14) F. Šorm, *Pure and Applied Chemistry*, **21**, 263 (1970).
15) *cf.*, G. Snatzke, *Tetrahedron*, **21**, 413 (1965).



of the 1,10-epoxide (**6**) itself shows a negative n—π^* Cotton effects as is expected from the octant diagram.[15] The stereostructures of the three epoxides (**6**, **7**, and **8**) have thus been elucidated. The present fermentation study has thus revealed a new type of bio-oxygenative transformation, the stereoselective epoxidation of isolated unsaturation in terpenoids having no asymmetric centers.

Since the amplitudes of the ORD curves of the diepoxides from different sources were not constant, the optical purities of the epoxides (**6**, **7**, and **8**) were thought to be doubtful. Then the 4,5-epoxide (**7**) was subjected to rearrangement under various conditions. As the results, it was found that treatment of the 4,5-epoxide (**7**) ($[\phi]_{330}$—680, $[\phi]_{276}$+410) with *p*-toluenesulfonic acid in benzene at —20° furnished procurcumenol (**9**) which is another sesquiterpenic constituent of this plant, *C. zedoaria*.[16] Comparison of the optical rotation ($[\alpha]_D$+36°) of procurcumenol thus obtained with that ($[\alpha]_D$+141°) of the natural procurcumenol (**9**) revealed that although the enzymatic epoxidation of germacrone proceeds stereoselectively, the selectivity is not complete.



In evaluating the fermentation of germacrone with *C. lunata*, which is known to bring about epoxidation of non-conjugated unsaturation in steroids, the presence of a component moving exactly like authentic epoxides in thin-layer chromatography (TLC) could not be demonstrated. Upon incubation with other microorganisms capable of causing the epoxidation of steroidal substrates such as *Curvularia lunata*, *Helicostylum piriforme*, and *Mucor griseocyanus*, formation of any of the epoxides was not similarly observed under the condition employed in the present work.

In connection with this transformation of the epoxide (**7**) to procurcumenol (**9**), the stereostructure of the natural procurcumenol which has been unknown, has thus been elucidated from the mechanism of the transannular cyclization as discussed below.

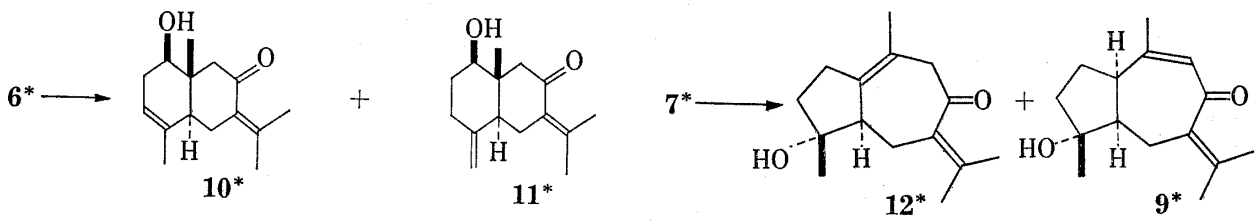
It is interesting to note that the absolute configurations of the epoxide rings in zederone (**2**) and the postulated intermediate (**4**) generated in a plant coincide with those in the 4,5-epoxide (**7**) and the 1,10-epoxide (**6**), respectively, formed by fermentation of germacrone.

Our next interests were directed towards the specificity of transannular cyclization of germacradienes and their epoxides in relation to the biosynthesis of the naturally occurring sesquiterpenoids of the selinane type and of the guaiane type since it is most probable that these bicarbocyclic sesquiterpenoids are derived from germacrane intermediates having ten-membered carbocyclic rings by enzymatic transannular cyclization. Transannular cyclization of germacradienes and their epoxides was first carried out on pyrethrosin by

16) H. Hikino, Y. Sakurai, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1605 (1968).

Barton and de Mayo[17] who obtained cyclopyrethrosin acetate, and was later performed by a number of investigators.[18–25] We have also carried out transannular cyclization of germacrone 1,10-epoxide (**6**) as an example whose C-1, C-10 position is first attacked by acid, and of germacrone 4,5-epoxide (**7**) as an example whose C-4, C-5 position is first attacked by acid.

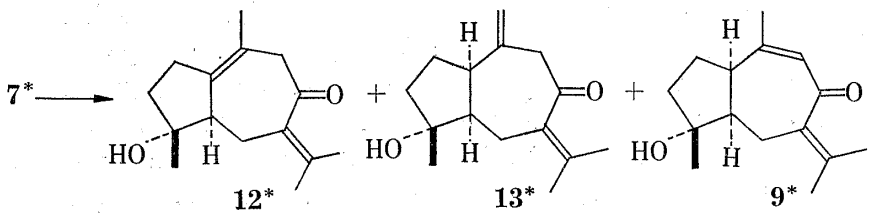
Treatment of germacrone 1,10-epoxide (**6***)[26] with boron trifluoride in ether gave two products. The less polar product possesses the composition $C_{15}H_{22}O_2$, the same as that of the original epoxide, and has the spectral properties indicating the presence of a hydroxyl (3400 cm^{-1}) and an α,β-unsaturated carbonyl (248 nm, 1680 cm^{-1}). In the NMR spectrum, signals due to a tertiary methyl (0.80 ppm), three vinyl methyls (1.68, 1.81, 2.04 ppm), a carbinyl hydrogen (3.51 ppm), and a vinyl hydrogen (5.30 ppm) are visible. Among the vinyl methyl signals, those at 1.81 and 2.04 ppm exhibited the solvent induced shifts on passing from carbon tetrachloride to benzene ($\delta_{CCl_4}-\delta_{C_6H_6}$) of +0.31 and −0.06 ppm, respectively. These values coincide with those (+0.26 and −0.11 ppm) for the vinyl methyls of pulegone,[27] demonstrating that although the 7(11)-en-8-one moiety in the original epoxide (**6**) is still retained in the product, the *s-trans* arrangement in the former has been converted into the *s-cis* arrangement in the latter. These data coupled with the degree of unsaturation of the molecule point to the bicarbocyclic nature and on the basis of the mechanism of the reaction, the product is concluded to have the structure **10***. The splitting pattern of the C-1 carbinyl hydrogen signal (doublet of doublets, $J_{1,2}=6$ and $J_{1,2'}=10$ Hz) supports the postulated structure. The more polar product has the same molecular formula $C_{15}H_{22}O_2$ and is shown by its spectral properties to possess a hydroxyl (3400 cm^{-1}) and an α,β-unsaturated carbonyl (250 nm, 1680 cm^{-1}). The NMR spectrum indicates the presence of a tertiary methyl (0.71 ppm), two vinyl methyls (1.80, 1.95 ppm), a carbinyl hydrogen (3.44 ppm), and a vinylidene (4.60, 4.80 ppm). The two vinyl methyl signals at 1.80 and 1.95 ppm showed the solvent shifts ($\delta_{CCl_4}-\delta_{C_6H_6}$) of +0.28 and −0.09 ppm, respectively, demonstrating that the 7(11)-en-8-one moiety should be in the *s-cis* arrangement. Combined evidence together with the bicarbocyclic nature deduced from the degree of unsaturation of the product and with the reaction mechanism lead to the conclusion that the second products represented by formula **11***. The splitting pattern of the carbinyl hydrogen (doublet of doublets, $J_{1,2}=5$ and $J_{1,2'}=$ 10 Hz) in the NMR spectrum of the more polar product again supports the structure.



17) D.H.R. Barton and P. de Mayo, *J. Chem. Soc.*, **1957**, 150.
18) M. Martin-Smith, P. de Mayo, S.J. Smith, J.B. Stenlake, and W.D. Williams, *Tetrahedron Letters*, **1964**, 2391.
19) R.V.H. Jonis and M.D. Sutherland, *Chem. Commun.*, **1968**, 1229.
20) N.R. Unde, S.V. Hiremath, G.H. Kulkarni, and G.R. Kelker, *Tetrahedron Letters*, **1968**, 4861.
21) K. Morikawa and Y. Hirose, *Tetrahedron Letters*, **1969**, 1229.
22) M.F.L'Homme, T.A. Geissman, H. Yoshioka, T.H. Porter, W. Renold, and T.J. Marbry, *Tetrahedron Letters*, **1969**, 3161.
23) R.W. Doskotch and F.S. El-Feraly, *J. Org. Chem.*, **35**, 1928 (1970).
24) S.S. Chandra, P. Shashikumark, and S.C. Bhattacharyya, *Indian J. Chem.*, **8**, 850 (1970).
25) J.K. Sutherland, *Tetrahedron*, **30**, 1651 (1974) and references cited therein.
26) The asterisked compounds are racemic and the formulas shown represent counterparts of the enantiomeric pairs.
27) C.J. Timmons, *Chem. Commun.*, **1965**, 576.

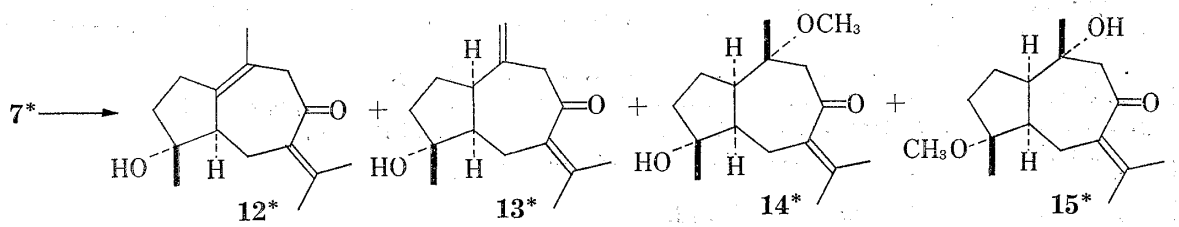
Treatment of germacrone 4,5-epoxide (**7***) with *p*-toluenesulfonic acid in benzene afforded two products. The less polar product analyzed for $C_{15}H_{22}O_2$ and is revealed to possess a hydroxyl (3450 cm^{-1}) and an α,β-unsaturated carbonyl (1670 cm^{-1}). In the NMR spectrum, the presence of a tertiary methyl (1.03 ppm) on oxygen bearing carbon, and three vinyl methyls (1.65, 1.80, 1.88 ppm) is indicated but a signal for a carbinyl hydrogen or a vinyl hydrogen is not detected. These data along with the reaction mechanism show that the product has the structure **12***. The more polar product was found to be identical with procurcumenol (**9***).

Treatment of germacrone 4,5-epoxide (**7***) with boron trifluoride in ether yielded three products. The least polar product has the composition $C_{15}H_{22}O_2$ and is shown to have a hydroxyl (3420 cm^{-1}) and an *s-cis* α,β-unsaturated carbonyl (1698, 1615 cm^{-1}), and a vinylidene (3080, 1646, 900 cm^{-1}). The NMR spectrum reveals the presence of a tertiary methyl (1.17 ppm) on an oxygen carrying carbon, two vinyl methyls (1.72, 1.81 ppm), and a vinylidene (4.68 ppm). Based on these data and the reaction mechanism, the product is deduced to be **13***. The second and the third products were revealed to be identical with the previous ketol (**12***) and procurcumenol (**9***), respectively.



Treatment of germacrone 4,5-epoxide (**7***) with boron trifluoride in methanol formed four products. Among these, the two less polar products were identified as the ketols (**12*** and **13***). The third product, $C_{16}H_{26}O_3$, is indicated to have a hydroxyl (3540 cm^{-1}), an *s-cis* α,β-unsaturated carbonyl (1662, 1605 cm^{-1}), two tertiary methyls (1.00, 1.11 ppm) on oxygen carrying carbon, two vinyl methyls (1.80, 1.86 ppm), and a methoxyl (3.10 ppm). The fourth product, $C_{16}H_{26}O_3$, is shown to possess a hydroxyl (3480 cm^{-1}), an *s-cis* α,β-unsaturated carbonyl (1676, 1627 cm^{-1}), two tertiary methyls (1.10, 1.32 ppm) on oxygen bearing carbon, two vinyl methyls (1.80, 1.90 ppm), and a methoxyl (3.06 ppm). On the basis of the above evidence, the two products are considered to be isomers. When the third product was treated with phosphorus oxychloride in pyridine the anhydro-derivative (**16***) was obtained. In the NMR spectrum of the anhydro-derivative (**16***), the signal due to a tertiary methyl (1.32 ppm) on oxygen carrying carbon, observed in the spectrum of the third product, is absent and instead signals attributable to a vinyl methyl (1.66 ppm) and a vinyl hydrogen (5.30 ppm) appear, indicating the formation of a trisubstituted ethylenic linkage during dehydration. The vinyl hydrogen signal, upon irradiation at the vinyl methyl signal, occurred as a doublet of doublets (J=4 and 2 Hz) which demonstrates that the ethylenic bond is adjacent to a methylene grouping. These findings coupled with the reaction mechanism show the third product to have the structure **14*** and consequently the fourth product to be **15***.

Summarizing the results obtained above together with the previous knowledge on the transannular cyclization of similar epoxides,[18,24,25] it can be concluded that the rearrangement



of germacra-1(10), 4-diene 1,10-epoxides gives only eudesmane derivatives having 6,6-membered rings, while rearranged products of 4,5-epoxides are all guaiane derivatives having 5,7-membered rings. This regurality seems to hold in the biogenesis of known sesquiterpenoids. Thus, there are a vast number of sesquiterpenoids of eudesmane type and of guaiane type in nature which are considered biogenetically to be arising from monoepoxides of germacradiene derivatives by opening of epoxide rings concomitant with transannular cyclization, but *in vitro* 1,10-epoxides give only the eudesmane derivatives and, on the other hand, 4,5-epoxides afford only the guaiane derivatives.

There are another type of sesquiterpenoids of eudesmane type and of guaiane type in nature which are thought to form directly from germacradiene derivatives by attack of acids (nucleophilic reagents) to double bonds concomitant with transannular cyclization. Again it is realized that biogenetically attack of acids to 1,10-double bonds gives eudesmane derivatives, while attack of acids to 4,5-double bonds affords guaiane derivatives. On the other hand, as far as the reaction is conducted chemically, acid-induced transannular cyclization of germacradienes yields only eudesmane derivatives[19–23,25] which is initiated by preferential attack of acids to the 1,10-double bonds.[28] Discussion about this specificity of the rearrangement will be made below.

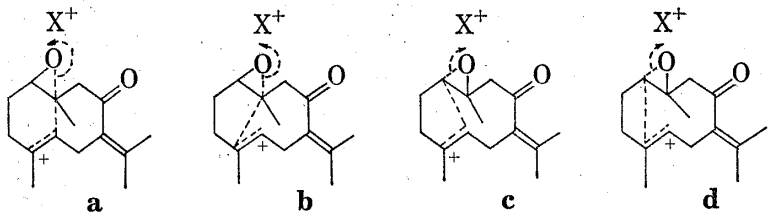
There are two problems in the transannular cyclization of germacradienes. The first one is the direction of the cyclization and its mechanism, and the second one is the stereochemistry of the cyclization products.

Concerning the first problem, the mechanism of the transannular cyclization of germacradienes, Brown and Sutherland[29] pointed out that the cyclization of germacra-1(10)E,4E,7(11)-triene and its 1,10-epoxide proceeds according to the Markownikoff's rule while the cyclization of germacratriene 4,5-epoxide involves the anti-Markownikoff addition followed by the Markownikoff addition. However, no detailed discussion about the mechanisms, and, in particular, no explanation for the inevitability of the occurrence of the anti-Markownikoff addition were made. Now, the reason for the proceeding of reactions against the Markownikoff's rule in certain cases must be first explained. A clue may be obtained from the results of X-ray analysis of a few germacradienes and their epoxides, such as germacratriene,[12] pregeijerene, shirodamol, and elephantol,[30] which indicate that E double bonds of germacradienes or even E double bonds of germacradiene monoepoxides are subjected to significant steric strain, and the 9-10-1-2 torsion angles and 4-5-6-7 torsion angles differ substantially from the value of 180° appropriate to an ideal unstrained double bonds. The principal source of the apparent twists in these four compounds being originating from out-of-plane displacement of the C=C double bonds, a fact which demonstrates the presence of a severe compression of electrons between certain opposed carbons. In the conformation of germacrone, the double bonds are also not planer, and the apparent twists in the E double bonds are the out-of-plane displacement of the bonds at C-5 and C-10. As a result, the C-1 and C-4 non-bonded intramolecular distance is distinctly short, 2.84Å, so that there must also be a severe compression. Therefore, it may be considered that conversion of sp^2 at a certain carbon into sp^3 would relieve the electron compression and this energy gain may possibly be a reason why the reaction proceeds against the Markownikoff's rule which must be unfavorable as it is. In these cases where the severe electron compression is present, the transannular cyclization would proceed readily in a concerted manner in order to dissolve the distortion. Provided that the reaction proceeds concertedly, the transannular cyclization of 1,10-epoxy-germacrone (**6**), as an example where a nucleophilic reagent attacks at the C-1, C-10 position, is expected to proceed through one of the active

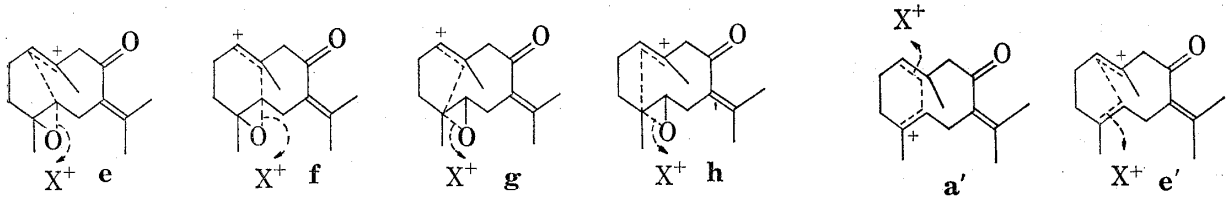
28) The exceptions are the transannular cyclization of bicyclogermacrene and germacra-1(10)E,4Z-dien-6-one which apparently involves the other factors to participate the reaction.[25]

29) E.D. Brown and J.K. Sutherland, *Chem. Commun.*, **1968**, 1060.

30) R.J. McClure and G.A. Sim, *Chem. Commun.*, **1970**, 128.



complexes **a**—**d**, and, in fact was revealed to follow only **a**. While the transannular cyclization of 4,5-epoxy-germacrone (**7**), as an example where a nucleophilic reagent attacks at the C-4, C-5, is considered to take place through one of the active complexes **e**—**h**, and, in reality,



was shown to proceed *via* only **e**. These facts must be explained by the thermodynamical stability of active complexes **a**—**h** generated during the course of reactions. Thus, in **b** and **d** carbonium ions remain at secondary carbons (**d** further requires an unfavorable four-membered ring formation) and in **c** the epoxy oxygen in removed from a tertiary carbon; consequently all must be thermodynamically more unstable than **a**. Therefore, in the case of 1,10-epoxides, the cyclization proceeds *via* **a** in full accordance with the Markownikoff's rule when the activation energy is the minimum. On the other hand, the active complex **h** follows from strict application of Markownikoff's rule so that it seems to be the electronically most stable one among **e**—**h**. However, it requires to form a four-membered ring which is sterically very unfavorable. In the remaining **e**—**g**, **f** and **g** is considered to be more unstable than **e**, because carbonium ions remain at secondary carbons. Hence, in the case of 4,5-epoxides, the reaction proceeds *via* **e** when the activation energy is thought to be the minimum.

As has been mentioned before, transannular cyclization of germacradienes, which was conducted by chemical reagents, give only eudesmane derivatives yielded by attack of acids to 1,10-double bonds but not guaiane derivatives generated by attack of acids to 4,5-double bonds. In order to explain this finding, Sutherland[25] postulated that transannular C–C bond formation is synchronous with C–X bond formation (X: a nucleophilic reagent), since nucleophilic reagents which do not lead to cyclization (such as peracid) show similar affinities for the 1,10- and 4,5-double bonds. However, we are in the opinion that the specificity of the transannular cyclization can be rationalized simply by the smaller activation energy in the transition state in proceeding *via* the active complex **a'** than *via* **e'**. It is worthy to note that, different from chemical reagent-conducted cyclization, enzyme-conducted transannular rearrangement of germacradienes in the biosynthesis of sesquiterpenoids results in the formation of guaiane derivatives as well as eudesmane derivatives.

The second problem is the stereochemistry of the cyclized products. Double bonds moieties of germacra-1(10)E, 4E-dienes and their epoxides adopt the conformations similar to that of A and transannular cyclization of these substances gives rise to *trans*-eudesmanes or *cis*-guaianes. On the basis of the accumulated results, it is concluded that the stereochemistry of the cyclization products is dependent upon the conformation of the starting cyclodeca-dienes.[25] The cyclization of cyclodeca-1Z,5E-diene was shown to afford a *cis*-decalin,[31] demonstrating that the reaction follows from the above regularity. Recently, it was reported

31) T.G. Trynham, C.R. Franzer, G. A. Knesel, and D.J. Northing, *J. Org. Chem.*, **32**, 3285 (1967).

that germacra1(10)E,4Z-dien-6-one 1,10-epoxide (**17**) on transannular cyclization yielded a *cis*-eudesmane derivative.[32] However, the conformation of the starting material (**17**) is not known so that the result of its transannular cyclization could not be taken as a conclusive evidence. Examination of the conformation of the sesquiterpenoid (**17**) by means of NMR spectroscopy was now carried out to reveal that an NOE is present between the C-15 methyl hydrogens and the C-5 vinyl hydrogen, a fact which indicates that these hydrogens are located in a *syn* relationship. After the establishment of the conformation, the cyclization product from the sesquiterpenoid (**17**) is expected to possess the *cis*-eudesmane structure which is in accordance with the actual result, providing further evidence confirming the above regularity.

Experimental[33]

Screening Procedure for Microbial Transformation of Germacrone——A 500 ml flask was charged with a *Corticium* synthetic medium (100 ml). The pH of the medium was adjusted to 6.8—7.0 with 1N NaOH, and the vessel and medium were sterilized at 120° for 20 min. After cooling to room temperature, the flask was inoculated with mycelia of *Cunninghamella blakesleeana*, *Curvularia lunata*, *Helicostylum piriforme*, or *Mucor griseocyanus*. The culture was shaken at 27° for a period of 4 days. A solution of germacrone (30 mg) in EtOH (1 ml) was added to each flask and the fermentation was continued for 3 or 6 days. The filtrate of the culture broth was then extracted with ether and the extract was evaporated to give a fermentation product which was subjected to TLC.

Fermentation of germacrone with *Cunninghamella blakesleeana*. The harvested fermentation product (4.8 g), obtained from germacrone (3.0 g) by action of *C. blakesleeana* for 3 days, was chromatographed over silica gel (70 g).

Elution with light petroleum–benzene (1: 1) and crystallization from light petroleum gave the recovered germacrone as colorless needles (180 mg), mp 53—54°. IR ν_{max}^{KBr} cm^{-1}: 1678, 1637 (enone), 1656 (double bond).

Elution with benzene and crystallization from light petroleum afforded the partially racemized 1,10-oxido-germacra-4,7(11)-dien-8-one (**6**) as colorless needles (27 mg), mp 63—64°. CD (*c* 0.231, EPA, 20°): $[\theta]_{325}$ −75; CD (*c* 0.231, EPA, −68°): $[\theta]_{327}$ −140, $[\theta]_{340}$ −100. Mass Spectrum *m/e*: 234 (M$^+$). UV λ_{max}^{EtOH} nm (log ε): 252 (3.57), 310 (2.70); IR ν_{max}^{KBr} cm^{-1}: 1680, 1665 (enone); NMR: Table I.

Successive elution with benzene–AcOEt (10: 1) and crystallization from light petroleum yielded the partially racemized 4,5-oxido-germacra-1(10),7(11)-dien-8-one (**7**) as colorless needles (183 mg), mp 80.5—81.5°. ORD (*c* 0.387, EtOH): $[\phi]_{330}$ −680. $[\phi]_{276}$ +410; CD (*c* 0.293, EPA, 20°): $[\theta]_{282}$ −81, $[\theta]_{291}$ −53, $[\theta]_{298}$ −118, $[\theta]_{304}$ −24, $[\theta]_{309}$ −122, $[\theta]_{314}$ −26, $[\theta]_{319}$ −78, $[\theta]_{322}$ −35, $[\theta]_{328}$ −50; CD (*c* 0.293, EPA, −68°) $[\theta]_{275}$ −50, $[\theta]_{298}$ −123, $[\theta]_{310}$ −110, $[\theta]_{319}$ −80. Mass Spectrum *m/e*: 234 (M$^+$). UV λ_{max}^{EtOH} nm (log ε): 240 (3.72), 310 (2.95); IR ν_{max}^{KBr} cm^{-1}: 1672, 1666 (enone); NMR: Table I.

Further elution with the same solvent and crystallization from AcOEt furnished 1,10; 4,5-dioxido-germacr-7(11)-en-8-one (**8**) as colorless needles (241 mg), mp 123.5—124.5°. ORD (*c* 0.223, EtOH): $[\phi]_{340}$ −15, $[\phi]_{280}$ −570; CD (*c* 0.223, EtOH): $[\theta]_{320}$ +80; CD (*c* 0.242, EPA, 20°): $[\theta]_{320}$ +75; CD (*c* 0.242, EPA, −68°): $[\theta]_{320}$ +94. Mass Spectrum *m/e*: 250 (M$^+$). UV λ_{max}^{EtOH} nm (log ε): 250 (3.68), 315 (2.30); IR ν_{max}^{KBr} cm^{-1}: 1673, 1643 (enone); NMR: Table I.

Oxidation of Germacrone with Perbenzoic Acid——To germacrone (300 mg) in $CHCl_3$ (10 ml) was added BzO_2H (210 mg) in $CHCl_3$ (5 ml) at 0°. After 30 min, isolation in the customary manner gave a product which was chromatographed over silica gel (20 g).

Elution with benzene and crystallization from light petroleum gave (±)-germacrone 1,10-epoxide as colorless needles (7 mg), mp 63—64°. IR ν_{max}^{KBr} cm^{-1}: 1680, 1665 (enone); NMR (CCl_4): 3H s at 1.19 ($C_{(15)}\underline{H}_3$), 3H br at 1.51 ($C_{(14)}\underline{H}_3$), 3H br at 1.65 ($C_{(12)}\underline{H}_3$), 3H s at 1.74 ($C_{(13)}\underline{H}_3$), 1H dd at 2.52, (J=2, 11, $C_{(1)}\underline{H}$), 1H dd at 4.95 (J=12, 6, $C_{(5)}\underline{H}$).

Successive elution with the same solvent and crystallization from light petroleum afforded (±)-germacrone 4,5-epoxide as colorless needles (173 mg), mp 80.5—81.5°. IR ν_{max}^{KBr} cm^{-1}: 1672, 1666 (enone); NMR ($CHCl_3$): 3H s at 1.02 ($C_{(14)}\underline{H}_3$), 3H br at 1.72 ($C_{(15)}\underline{H}_3$), 6H s at 1.81 ($C_{(12)}\underline{H}_3$, $C_{(13)}\underline{H}_3$), 1H dd at 2.42 (J=2, 11, $C_{(5)}\underline{H}$), 1H dd at 5.20 (J=7, 7, $C_{(1)}\underline{H}$).

Elution with benzene–AcOEt (10: 1) and crystallization from light petroleum yielded (±)-germacrone 1,10; 4,5-diepoxide as colorless needles (36 mg), mp 123—124°. IR ν_{max}^{KBr} cm^{-1}: 1673, 1643, (enone); NMR (CCl_4): 3H s at 1.08 ($C_{(14)}H_3$), 3H s at 1.37 ($C_{(15)}\underline{H}_3$), 3H s at 1.79 ($C_{(12)}\underline{H}_3$), 3H s at 1.87 ($C_{(13)}\underline{H}_3$).

32) M. Iguchi, M. Niwa, and S. Yamamura, *Chem. Commun.*, **1972**, 689.

33) All mp are uncorrected. NMR spectra were determined at 60 MHz unless indicated otherwise. Chemical shifts are given in ppm from internal tetramethyl silane (TMS) and coupling constants (J) in Hz. Abbreviations: s=singlet, d=doublet, dd=doublet of doublets, br=broad peak.

The epoxides thus obtained were identified as the epoxides (**6**, **7**, and **8**) formed by fermentation of germacrone, respectively, by comparison of TLC, IR and NMR spectra.

Oxidation of Germacrone 1,10-Epoxide with Perbenzoic Acid——To germacrone 1,10-epoxide (**6**) (8 mg) in $CHCl_3$ (1 ml) was added BzO_2H (21 mg) in $CHCl_3$ (0.5 ml) at 0° and the mixture was set aside for 2 hr. Ether extraction and crystallization from light petroleum gave germacrone 1,10;4,5-diepoxide (antipode of **8**) as colorless needles (7 mg), mp 123.5—124.5°. ORD (*c* 0.306, EtOH): $[\phi]_{340}$ +30, $[\phi]_{280}$ −620. Identification was carried out by mixed mp and comparison of TLC and IR spectra.

Oxidation of Germacrone 4,5-Epoxide with Perbenzoic Acid——To germacrone 4,5-epoxide (**7**) (20 mg) in $CHCl_3$ (1 ml) was added BzO_2H (42 mg) in $CHCl_3$ (1 ml) at 0°. After 2 hr, the mixture was worked up as usual and the product crystallized from light petroleum to furnish germacrone 1,10;4,5-diepoxide (**8**) as colorless needles (17 mg), mp 123—124°. ORD (*c* 0.337, EtOH): $[\phi]_{340}$ −10, $[\phi]_{280}$ −220. IR ν_{max}^{KBr} cm^{-1}: 1673, 1643 (enone); NMR (CCl_4): 3H s at 1.08, 3H s at 1.37, 3H s at 1.79, 3H s at 1.87. The identity was confirmed by mixed mp and comparison of TLC, IR and NMR spectra.

Preparation of Germacrone-silver Nitrate Adduct——To germacrone (96 mg) in *n*-hexane (0.5 ml) was added 50% $AgNO_3$ soln (0.5 ml). After standing for 3 hr, the deposited crystals were washed with *n*-hexane (3 ml) and then crystallized from EtOH to furnish germacrone-silver nitrate adduct as colorless prisms (20 mg), mp 174.5—175° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 1675 (enone), 1370 (nitrate ion).

Crystal Analysis of Germacrone-silver Nitrate Adduct Performed by Drs. K. Kamiya, Y. Wada, and M. Nishikawa——$C_{15}H_{22}O \cdot AgNO_3$, M=388.2, orthorhombic, a=8.91, b=23.93, c=7.55 Å, U=1610 Å^3, Z=4, Dc=1.60 g cm^{-3}, $F(000)$=792. Space group $P2_12_12_1$. Absorption coefficient for M_o–K_α radiation (λ=0.7107 Å) μ=12.4 cm^{-1}.

The cell parameters were obtained from oscillation and Weissenberg photographs taken with Cu-Kα radiation (λ=1.5418 Å), and the space group was determined uniquely from the systematic absences in the X-ray spectra. For the intensity measurements the *hk*0-*hk*6 layers were measured by a linear diffractometer using M_o–K_α radiation. The intensities were corrected for Lorentz and polarization factors. No correction was made for absorption.

The measurements provided a total of 1496 independent $|Fo|$ values by Wilson's method. Initial co-ordinates for the silver ion, (0.25, 0.20, 0.03), were readily derived from the three-dimensional Patterson synthesis. A minimum function based on these co-ordinates was calculated and 4 intense peaks in the resulting map were assigned to NO_3 group. The whole structure of germacrone silver nitrate adduct was derived from the alternative application of Fourier syntheses and least-squares treatments.

The atomic co-ordinates and isotropic temperature factors were refined by least-squares treatments, the final unweighted residual factor was 0.126. The atomic parameters are listed in Table II. The perspective view of the molecule in the crystal can be seen in Fig. 1. Bond distances and angles are given in Tables III and IV, respectively.

Table II. Atomic Coordinates and Temperature Factors for Germacrone-silver Nitrate Adduct

Atom	x/a	y/b	z/c	b
Ag	0.2370	0.1968	0.0089	3.97
N	0.0885	0.3076	0.0263	3.75
O (1)	0.1289	0.0305	0.5206	3.53
O (2)	0.0030	0.2725	0.9924	4.60
O (3)	0.2218	0.3004	0.0016	4.57
O (4)	0.0298	0.3537	0.0507	4.54
C (1)	0.2150	0.1706	0.6855	3.82
C (2)	0.1319	0.2242	0.5988	4.48
C (3)	0.1738	0.2395	0.4183	4.37
C (4)	0.1427	0.1842	0.3181	2.77
C (5)	0.2629	0.1488	0.2933	4.27
C (6)	0.2793	0.0831	0.2709	5.12
C (7)	0.3399	0.0610	0.4528	4.42
C (8)	0.2354	0.0545	0.5795	3.93
C (9)	0.2439	0.0799	0.7707	3.46
C (10)	0.1545	0.1339	0.7661	4.39
C (11)	0.4973	0.0544	0.4742	4.34
C (12)	0.0529	0.4727	0.3531	5.50
C (13)	0.1175	0.4350	0.6796	6.34
C (14)	0.9872	0.1597	0.2508	8.24
C (15)	0.4856	0.3737	0.2064	3.52

Table III. Bond Lengths for Germacrone-silver Nitrate Adduct

N —O (2)	1.17	C (6)—C (7)	1.52
N —O (3)	1.22	C (7)—C (8)	1.36
N —O (4)	1.23	C (7)—C (11)	1.38
O (1)—C (8)	1.23	C (8)—C (9)	1.56
C (1)—C (2)	1.57	C (9)—C (10)	1.51
C (2)—C (3)	1.46	C (10)—C (1)	1.21
C (3)—C (4)	1.56	C (10)—C (15)	1.53
C (4)—C (5)	1.36	C (11)—C (12)	1.57
C (4)—C (14)	1.55	C (11)—C (13)	1.58
C (5)—C (6)	1.57		

Table IV. Bond Angles for Germacrone-silver Nitrate Adduct

O (2)—N —O (3)	119	C (8)—C (7)—C (11)	127
O (2)—N —O (4)	115	C (7)—C (8)—C (9)	127
O (3)—N —O (4)	124	C (7)—C (8)—O (1)	110
C (1)—C (2)—C (3)	121	O (1)—C (8)—C (9)	123
C (2)—C (3)—C (4)	101	C (8)—C (9)—C (10)	109
C (3)—C (4)—C (5)	119	C (9)—C (10)—C (1)	113
C (3)—C (4)—C (14)	129	C (9)—C (10)—C (15)	114
C (5)—C (4)—C (14)	111	C (10)—C (1)—C (2)	128
C (4)—C (5)—C (6)	135	C (1)—C (10)—C (15)	125
C (5)—C (6)—C (7)	108	C (7)—C (11)—C (12)	117
C (6)—C (7)—C (8)	114	C (7)—C (11)—C (13)	126
C (6)—C (7)—C (11)	120	C (12)—C (11)—C (13)	117

Reaction of Germacrone 1,10-Epoxide with Boron Trifluoride in Ether——To germacrone 1,10-epoxide (**6***) (130 mg) in ether (20 ml) was added 45% BF_3 etherate (7 ml) at −20°. The mixture was set aside for 1 hr and worked up in the customary way to give a product (130 mg) which was submitted to silica gel chromatography.

Elution with benzene–AcOEt (5: 1) yielded 1-hydroxy-eudesma-3,7(11)-dien-8-one (**10***) as a colorless oil (40 mg). Mass Spectrum *m/e*: 234 (M^+). UV λ_{max}^{EtOH} nm (log ε): 248 (3.76), 295 (3.32); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3400 (OH), 1680 (enone); NMR (CCl_4): 3H s at 0.80 ($C_{(15)}\underline{H}_3$), 3H br at 1.68 ($C_{(14)}\underline{H}_3$), 3H s at 1.81 ($C_{(12)}\underline{H}_3$), 3H s at 2.04 ($C_{(13)}\underline{H}_3$), 1H dd at 3.51 (J=6, 10, $C_{(1)}\underline{H}$), 1H dd at 5.30 (J=4, 6, $C_{(3)}\underline{H}$); NMR (C_6H_6): 3H s at 0.80 ($C_{(15)}\underline{H}_3$), 6H s at 1.50 ($C_{(12)}\underline{H}_3$, $C_{(14)}\underline{H}_3$), 3H s at 2.10 ($C_{(13)}\underline{H}_3$), 1H m at 3.21 ($C_{(1)}\underline{H}$), 1H m at 5.15 ($C_{(3)}\underline{H}$).

Successive elution with the same solvent gave 1-hydroxy-eudesma-4,(14),7(11)-dien-8-one (**11***) as a colorless oil (35 mg). Mass Spectrum *m/e*: 234 (M^+). UV λ_{max}^{EtOH} nm (log ε): 250 (3.54), 290 (3.26); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3400 (OH), 1680 (enone); NMR (CCl_4): 3H s at 0.71 ($C_{(15)}\underline{H}_3$), 3H s at 1.80 ($C_{(12)}\underline{H}_3$), 3H s at 1.95 ($C_{(13)}\underline{H}_3$), 1H dd at 3.44 (J=5, 10, $C_{(1)}\underline{H}$), two 1H br's 4.60, 4.80 ($C_{(14)}\underline{H}_2$); NMR (C_6H_6): 3H s at 0.71 ($C_{(15)}\underline{H}_3$), 3H s at 1.52 ($C_{(12)}\underline{H}_3$), 3H s at 2.04 ($C_{(13)}\underline{H}_3$), 1H m at 3.00 ($C_{(1)}\underline{H}$), two 1H br's at 4.48, 4.70 ($C_{(14)}\underline{H}_2$).

Reaction of Germacrone 4,5-Epoxide with *p*-Toluene Sulfonic Acid in Benzene——Germacrone 4,5-epoxide (**7**) (346 mg) and *p*-TsOH (100 mg) in benzene (15 ml) were made to react at −20° for 20 min. After working up in the usual manner, the product (338 mg) was chromatographed over silica gel (50 g).

Elution with benzene–AcOEt (5: 1) and crystallization from AcOEt gave 4-hydroxy-guaia-1 (10), 7(11)-dien-8-one (**12**) as colorless needles (235 mg), mp 88—89°. Mass Spectrum *m/e*: 234 (M^+). UV λ_{max}^{EtOH} nm (log ε): 251 (3.78); IR ν_{max}^{KBr} cm^{-1}: 3490 (OH), 1674, 1619 (enone); NMR (CCl_4): 3H s at 1.03 ($C_{(14)}\underline{H}_3$), 3H s at 1.65 ($C_{(15)}\underline{H}_3$), 3H s at 1.80 ($C_{(12)}\underline{H}_3$), 3H s at 1.88 ($C_{(13)}\underline{H}_3$).

Successive elution with benzene–AcOEt (3: 1) afforded a product (42 mg) which was rechromatographed over alumina (10 g). Elution with benzene–AcOEt (2: 1) furnished procurcumenol (**9**) as a colorless oil (18 mg). $[\alpha]_D$ +36° (*c* 1.4, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3420 (OH), 1630 (dienone); NMR (CCl_4): 3H s at 1.16 ($C_{(14)}\underline{H}_3$), 6H s at 1.73 ($C_{(12)}\underline{H}_3$, $C_{(15)}\underline{H}_3$), 3H s at 1.88 ($C_{(13)}\underline{H}_3$), 1H br at 5.71 ($C_{(9)}\underline{H}$), which was identified by IR and NMR spectra as the natural procurcumenol (disregard of the optical rotation).

Reaction of Germacrone 4,5-Epoxide with Boron Trifluoride in Ether——To germacrone 4,5-epoxide (**7***) (320 mg) in ether (10 ml) was added BF_3 etherate (0.5 ml) at −20°. The mixture was left standing for 15 min and worked up as usual. The product (313 mg) was chromatographed over silica gel (30 g).

Elution with benzene–AcOEt (10: 1) and crystallization from AcOEt furnished 4-hydroxy-guaia-7(11), 10(15)-dien-8-one (**13***) as colorless needles (28 mg), mp 69—70°. Mass Spectrum *m/e*: 234 (M^+). UV λ_{max}^{EtOH} nm (log ε): 230 (3.96); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1698, 1615 (enone), 3080, 1647, 900 (vinylidene); NMR (CCl_4): 3H s at 1.17 ($C_{(14)}\underline{H}_3$), 3H s at 1.72 ($C_{(12)}\underline{H}_3$), 3H s at 1.81 ($C_{(13)}\underline{H}_3$), 2H br at 4.68 ($C_{(15)}\underline{H}_2$).

Successive elution with benzene–AcOH (10: 1) and crystallization from AcOEt gave 4-hydroxy-guaia-1(10), 7(11)-dien-8-one (**12**) as colorless needles (148 mg), mp 88—89°. IR ν_{max}^{KBr} cm^{-1}: 3490 (OH), 1674, 1619 (enone); NMR (CCl_4): 3H s at 1.03, 3H s at 1.65, 3H s at 1.80, 3H s at 1.89. The identity with 4-hydroxy-guaia-1(10),7(11)-dien-8-one obtained from germacrone 4,5-epoxide by *p*-TsOH-benzene was corroborated by usual criteria.

Further elution with benzene–AcOEt (2: 1) and rechromatography over alumina afforded procurcumenol (**9***) as a colorless oil (7 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3420 (OH), 1630 (dienone); NMR (CCl_4): 3H s at 1.16, 6H s at 1.73, 3H s at 1.88, 1H br at 5.71. Identification was performed by usual criteria.

Reaction of Germacrone 4,5-Epoxide with Boron Trifluoride in Methanol——Germacrone 4,5-epoxide (**7***) (830 mg) and 45% BF_3 etherate (12 ml) in MeOH (30 ml) were allowed to react at −20° for 20 min. After isolation in the customary manner, the product (810 mg) was chromatographed over silica gel (80 g).

Elution with benzene–AcOEt (5: 1) and crystallization from AcOEt gave 4-hydroxy-guaia-7(11), 10(15)-dien-8-one (**13***) as colorless needles (48 mg), mp 69—70°. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1698, 1615 (enone), 3080, 1647, 900 (vinylidene); NMR (CCl_4): 3H s at 1.17, 3H s at 1.72, 3H s at 1.81, 2H br at 4.68.

Successive elution with the same solvent and crystallization from AcOEt afforded 4-hydroxy-guaia-1(10), 7(11)-dien-8-one (**12***) as colorless needles (230 mg), mp 88—89°. IR ν_{max}^{KBr} cm^{-1}: 3490 (OH), 1674, 1619 (enone); NMR (CCl_4): 3H s at 1.03, 3H s at 1.65, 3H s at 1.80, 3H s at 1.88.

Elution with benzene–AcOEt (3: 1) and crystallization from AcOEt yielded 4-hydroxy-10-methoxy-guai-7(11)-en-8-one (**14***) as colorless needles (146 mg), mp 93—94°. Mass Spectrum *m/e*: 266 (M^+). UV λ_{max}^{EtOH} nm (log ε): 253 (3.89); IR ν_{max}^{KBr} cm^{-1}: 3540 (OH), 1662, 1605 (enone); NMR (CCl_4): 3H s at 1.00 ($C_{(14)}\underline{H}_3$), 3H s at 1.11 ($C_{(15)}\underline{H}_3$), 3H s at 1.80 ($C_{(12)}\underline{H}_3$), 3H s at 1.86 ($C_{(13)}\underline{H}_3$), 3H s at 3.10 ($C\underline{H}_3O$-).

Subsequent elution with the same solvent and crystallization from AcOEt furnished 10-hydroxy-4-methoxy-guai-7(11)-en-8-one (**15***) as colorless needles (25 mg), mp 101—103°. Mass Spectrum *m/e*: 266 (M^+). UV λ_{max}^{EtOH} nm (log ε): 250 (3.88); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3480 (OH), 1676, 1627 (enone); NMR (CCl_4): 3H s at 1.10 ($C_{(14)}\underline{H}_3$), 3H s at 1.32 ($C_{(15)}\underline{H}_3$), 3H s at 1.80 ($C_{(12)}\underline{H}_3$), 3H s at 1.90 ($C_{(13)}\underline{H}_3$), 3H s at 3.06 ($C\underline{H}_3O$-).

Dehydration of 4-Hydroxy-10-methoxy-guai-7(11)-en-8-one with Phosphorus Oxychloride in Pyridine——To 4-hydroxy-10-methoxy-guaienone (**14***) (50 mg) in pyridine (1 ml) was added $POCl_3$ (0.2 ml) at 0°. The mixture was left standing at 5° overnight. Working up in the usual way to give a product (42 mg) which was chromatographed over $AgNO_3$-inpregnated silica gel (10%; 3 g). Fraction eluted with benzene furnished 10-methoxy-guaia-3,7(11)-dien-8-one (**16***) as a colorless oil (18 mg). UV λ_{max}^{EtOH} nm (log ε): 250 (3.59), 290 (2.26). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1680, 1620 (enone); NMR (CCl_4, 100 MHz): 3H s at 1.08 ($C_{(15)}\underline{H}_3$), 3H d at 1.66 (J = 1, $C_{(14)}\underline{H}_3$), 6H s at 1.73 ($C_{(12)}\underline{H}_3$, $C_{(13)}\underline{H}_3$), 3H s at 3.15 ($C\underline{H}_3O$–), 1H m at 5.30 ($C_{(3)}\underline{H}$).

NOE Measurements on Germacra-1(10)E,4Z-dien-6-one 1,10-epoxide——The 100 MHz spectra were taken with a JEOL PS-100 NMR spectrometer for *ca.* 10% (w/v) carefully degassed solution of germacra-1(10)E,4Z-dien-6-one 1,10-epoxide (17) in CCl_4 and C_6D_6 in the frequency swept and external water-locked mode. NOE experiments were carried out by measuring the integrated intensities of a signal with and without irradiation of the second r.f. field at the resonance frequency of another signal, more than 3 times, with a sweep rate of 0.2 Hz/sec, and the enhancement was determined in %. The results are shown in Table V.

Table V. The Nuclear Overhauser Effects (Increases in Integrated Signal Intensities in %) for Germacra-1(10)E, 4Z-dien-6-one 1,10-epoxide

Irradiated signal	Observed signal		
	H-1	H-5	H-7
H-14	2[a]	26[a]	12[a]
	0[b]		
H-15		3[a]	
		5[b]	

a) in CCl_4 b) in C_6D_6

Acknowledgement We are indebted to Dr. S. Yamamura, Meijo University, for the gift of germacra-1(10)E,4Z-dien-6-one 1,10-epoxide. Thanks are also due to Dr. Nishikawa, Research Laboratories, Takeda Chemical Industries, Ltd., for the X-ray chrystallographic analysis to Dr. K. Kuriyama, Shionogi Research Laboratory, for measurements of the ORD data, and to Research Laboratories, Takeda Chemical Industries, Ltd., Research Laboratories, Sankyo & Co., Ltd., Research Laboratory, Yoshitomi Pharmaceutical Co., Ltd., and Analytical Laboratories, this Institute, for determination of mass and NMR spectra, and microanalysis.