HETEROCYCLES, Vol. 53, No. 6, 2000, pp. 1269 - 1283, Received, 11th January, 2000

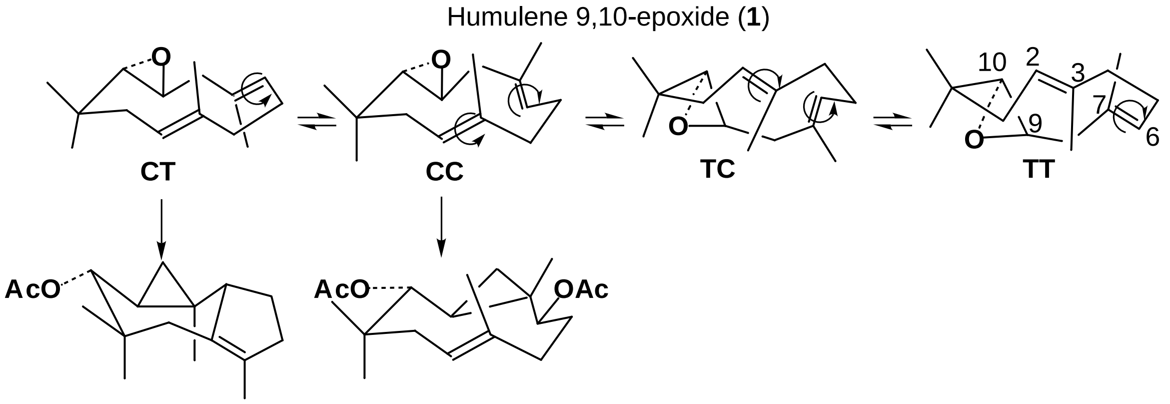
CONFORMATION AND CONFIGURATION OF HUMULENE DI- AND TRIEPOXIDES GENERATED FROM FOUR POSSIBLE CONFORMATIONS OF HUMULENE 9,10-EPOXIDE

Kiyoharu Hayano* and Katsura Mochizuki†

Chemistry Laboratory, Hokkaido College of Education, Ainosato, Sapporo 002-8502, Japan, †Department of Chemistry, Yokohama City University, Kanazawa-ku, Yokohama, 236-0027, Japan

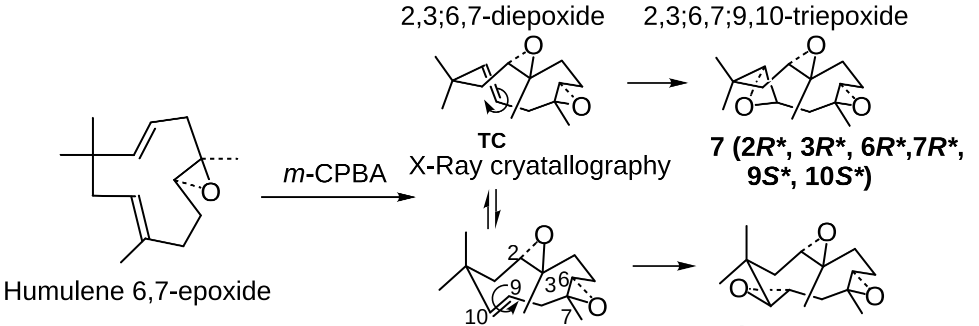
Abstract-The complete reaction of humulene 9,10-epoxide (**1**) with *m*-CPBA produced a hitherto unknown 2*R**, 3*R**, 6*S**, 7*S**, 9*S**, 10*S**-humulene 2,3;6,7;9,10-triepoxide (**6**) together with three known triepoxides (**7**, **8** and **9**) in the ratio of **6** : **7** : **8** : **9** = 1.3 : 11.5 : 22.6 : 64.6. The configuration of **6** was determined by X-Ray crystallography. Intermediate products of the epoxidation reaction, two humulene 6,7;9,10- {(2*E*)-6*R**, 7*R**, 9*S**, 10*S** (**2**) and (2*E*)-6*S**, 7*S**, 9*S**, 10*S** (**3**)} and two 2,3;9,10-diepoxides {(6*E*)-2*S**, 3*S**, 9*S**, 10*S** (**4**) and (6*E*)-2*R**, 3*R**, 9*S**, 10*S** (**5**)} were also produced in the ratio of **2** : **3** : **4** : **5** = 41 : 18 : 35 : 6), and these main conformations (CT, CC, CT and TC) were first determined by X-Ray crystallography (for **3** and **4**) and NMR spectroscopy (for **2** and **5**). The triepoxide (**6**) maintained the configuration corresponding to the less stable TT conformation of **1** and the minor TT conformation of **3** and **5**.

Humulene 9,10-epoxide {(2*E*, 6*E*)-9*S**, 10*S**-9,10-epoxy-3,7,11,11-tetramethylcycloundeca-2,6-diene (**1**)} is an important compound for generating the humulenic cation (9-position) during the biogenetic-like synthesis of sesquiterpene. It has been proposed that the epoxide (**1**) can maintain four conformations, TT, TC, CC, and CT, due to the rotation of the 2,3- and 6,7-double bond planes[1] as shown in Scheme 1. Actually, *in vitro*, two more stable CC and CT conformations of **1** were selectively frozen in two transannular cyclization products from which were synthesized the natural products, bicyclohumulenone and africanol,[2] respectively. However, the conformation of **1** has not been experimentally investigated and none of the compounds that originated from the residual conformations (TT and TC) of **1** has been produced.



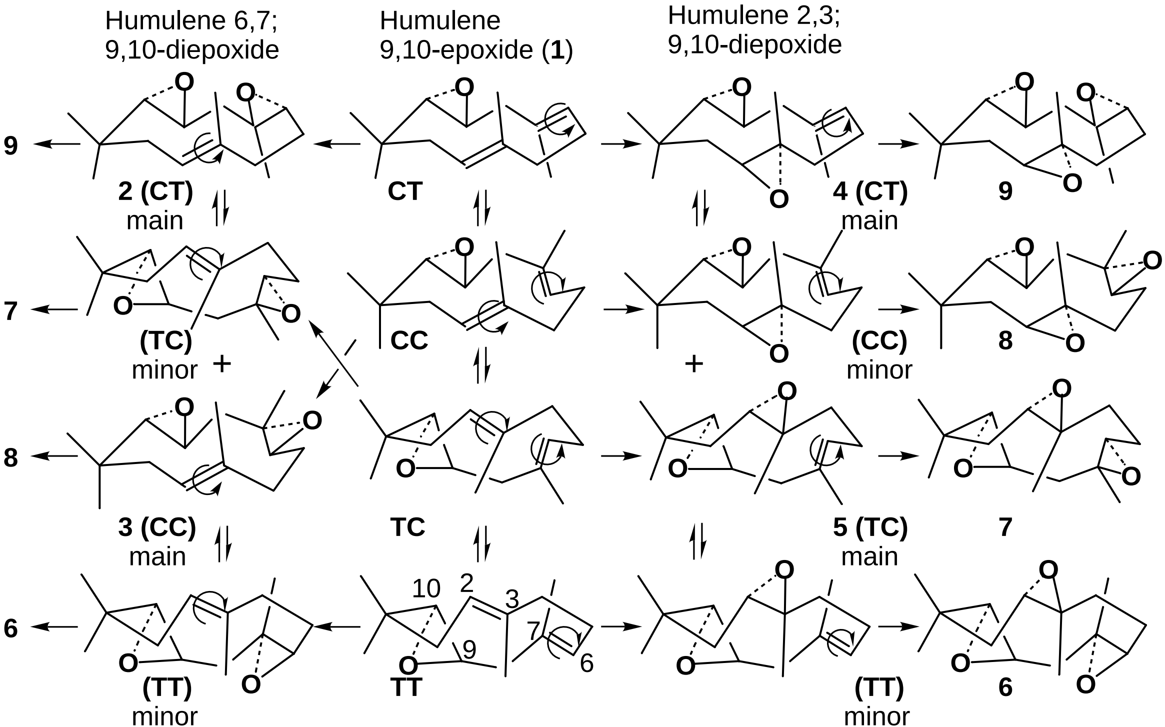
Scheme 1. Four possible conformations of **1**. C and T denote crossed and parallel arrangements of 6,7- and 9,10-bonds against the 2,3-bond, respectively. For example, first T and second T in TT represent parallel and parallel to that of the 9,10- and 2,3-bonds, and the 6,7- and 2,3-bonds, respectively.

Recently, we reported[3] the epoxidation of humulene 6,7-epoxide, the positional isomer of **1**. The epoxidation of the free rotating 2,3- and/or 9,10-double bond planes of the 6,7-epoxide resulted in the frozen of interconversion among the conformations assumed for the starting humulene epoxide. The thorough conformation and configuration analyses of the epoxidation products allowed us to estimate the possible conformation of the original epoxide, as well as revealing the conformation of the new diepoxide and configuration of the new triepoxides. Indeed, two new 2,3;6,7;9,10-triepoxdies that originated from the TC and CC conformations of the diepoxide were isolated, and we could experimentally suggest that the TC conformation should exist in the 6,7-epoxide and that the 2,3;6,7-diepoxide should be in equilibrium between the TC and CC conformations (Scheme 2).



Scheme 2

In the present study, the epoxidation of humulene 9,10-epoxide (**1**) was carried out and the main conformations and configurations of the di- and triepoxides were carefully investigated. As a result, the main CT and CC conformations of the two isolated humulene 6,7;9,10-diepoxides (**2** and **3**) in solution and a crystal, and the main CT and TC conformations of the two isolated humulene 2,3;9,10-diepoxides (**4** and **5**) in a crystal and solution were shown by X-Ray crystallography and NMR spectroscopy, respectively. Furthermore, the epoxidation reaction of **2**, **3**, **4** and **5** produced the 2,3;6,7;9,10-triepoxides (**7**, **6**, **8** and **6**) that originated from these minor TC, TT, CC and TT conformations together with **9**, **8**, **9** and **7** derived from these main conformations, respectively (Scheme 3). The triepoxide (**6**) was first isolated here and its configuration was determined by X-Ray crystallography.



Scheme 3. The epoxidation reaction of **1**.

The epoxidation of one double bond in humulene 9,10-epoxide (**1**) with *m*-CPBA at 0 ˚C under an argon atmosphere and then chromatographic separation gave an oily mixture (97%) of **2**, **3**, **4** and **5**. The ratio of (**2** and **3**) : (**4** and **5**) = 59 : 41 was based on the HPLC peak areas. The mixture was separated into an oily mixture (48.4 % from **1**) of two 6,7;9,10-diepoxides (**2** and **3**) and a crystalline mixture (33.6 % from **1**) of two 2,3;9,10-diepoxides (**4** and **5**) by HPLC. Furthermore, separation of the two mixtures (**2** : **3** = 69 : 31 and **4** : **5** = 85 : 15, ratios were calculated from the HPLC peak areas) by HPLC gave **2** (30.7 % from **1**), **3** (14.4 % from **1**), **4** (26.3 % from **1**) and **5** (4.6 % from **1**). The CC and CT conformations in the crystals of **3** and **4** were determined by X-Ray crystallography of these single crystals (Figures 1 and 2). The fractional atomic coordinates and the bond lengths and angles of **3** and **4** are listed in Tables 1 and 2.

The HRMS of the oily and crystalline diepoxides (**2** and **5**) showed the same molecular formula, $C_{15}H_{24}O_2$, as that of **3** and **4**. The partial structures of **2** and **5** revealed using the COSY (H-H and C-H)

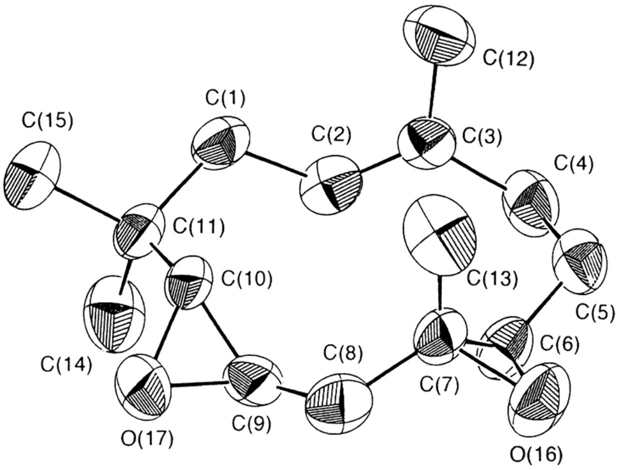


Figure 1. Perspective view of CC conformational humulene 6,7;9,10-diepoxide (**3**).

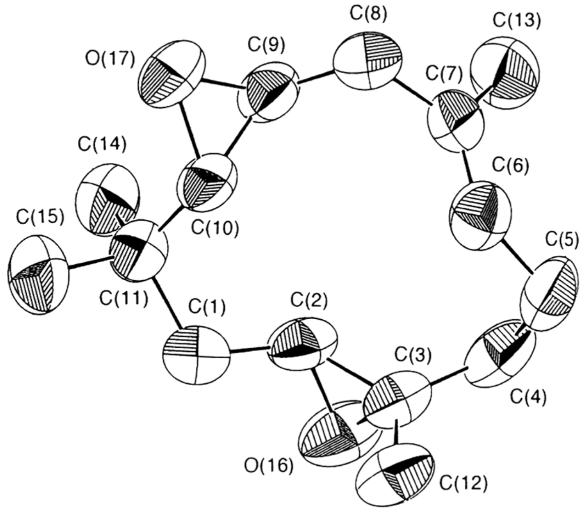


Figure 2. Perspective view of CT conformational humulene 2,3;9,10-diepoxide (**4**).

spectra were the same as those of **3** and **4**, respectively (Figures 3 and 4), although these diepoxides (**2** and **5**) differed from **3** and **4** in the ^{1}H and ^{13}C NMR spectra. Based on the above results, it was suggested that **2** and **5** were stereoisomers of **3** and **4**, respectively. Moreover, the NOESY spectra of **2** and **5** showed results of NOE as depicted in Figures 3 and 4, therefore, these structures and main conformations are represented as **2** (CT) and **5** (TC), respectively (Figures 3 and 4).

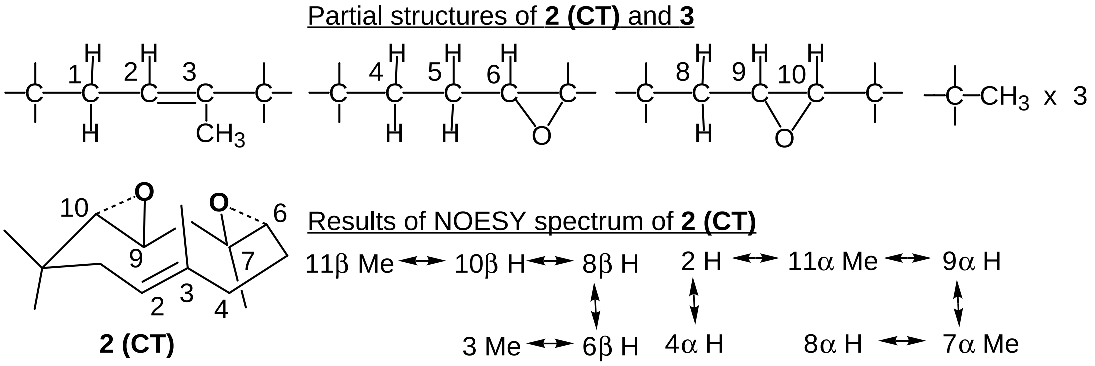


Figure 3. Partial structures of **2 (CT)** and **3**, and results of NOESY spectrum of **2 (CT)**.

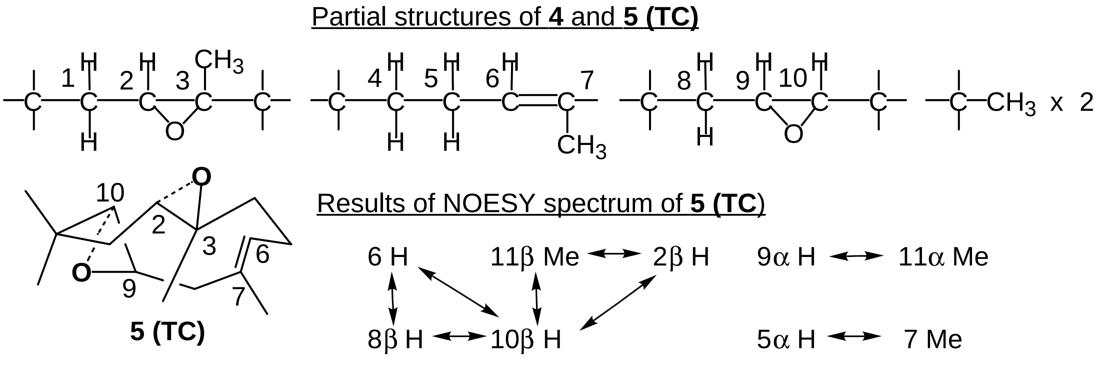
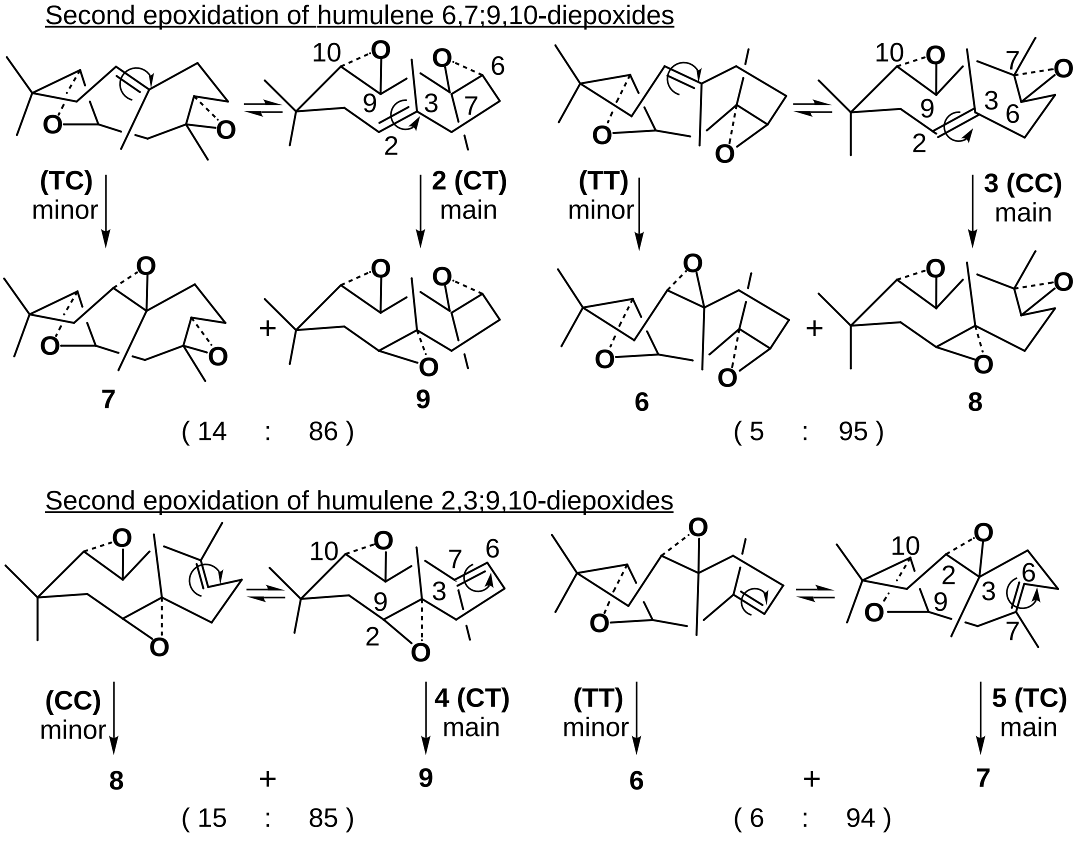


Figure 4. Partial structures of **4** and **5 (TC)**, and results of NOESY spectrum of **5 (TC)**.

The formation of two stereoisomeric 6,7;9,10- (**2** and **3**) and 2,3;9,10-diepoxides (**4** and **5**) from **1** indicates the rotation of the 6,7- and 2,3-double bond planes in **1**. The CC conformation of **1** was determined to be **3** of the 6,7;9,10-diepoxide, and is not the 2,3;9,10-diepoxide due to the stability of the main CT conformation in **4**. Similarly, the TC conformation of **1** is confirmed as **5** of the 2,3;9,10-diepoxide, but is not in the 6,7;9,10-diepoxide because of the stability of the main CT conformation in **2**. The main conformation of CT and CC in **2** and **3**, and the CT and TC in **4** and **5** shows the possible CT, CC and TC conformations of **1**. It is also thought that the main CC and TC conformations of **3** and **5** are produced from **1** by the rotation of the 2,3- and 6,7-double bond planes of the minor TT conformation of **3** and **5** that originated from the less stable TT conformation of **1**, respectively. Therefore, the above result strongly suggests an equilibrium between the CT, CC and TC conformations in **1**, together with the possible existence of the TT conformation. Moreover, it is thought that the diepoxdes (**2**, **3**, **4** and **5**) are in equilibrium between the main conformations (CT, CC, CT and TC) and the minor conformations (TC, TT, CC and TT), respectively (Scheme 4).

The epoxidation of the residual double bond in the above pure diepoxides (**2**, **3**, **4** and **5**) with *m*-CPBA under an argon atmosphere, followed by chromatographic separation, quantitatively yielded a triepoxide



Scheme 4. Second epoxidation products of **2**, **3**, **4** and **5**.

mixture of **7** and **9** (**7** : **9** = 14 : 86), **6** and **8** (**6** : **8** = 5 : 95), **8** and **9** (**8** : **9** = 15 : 85) and **6** and **7** (**6** : **7** = 6 : 94), respectively (Scheme 4). The ratio of these mixtures was calculated based on the HPLC peak areas. Recrystallization of the mixture of **6** and **8**, and **6** and **7** from benzene, combined with the HPLC separation of these filtrates, produced **6** (4.7 % from **3**) and **8** (93.8 % from **3**), and **6** (4.8 % from **5**) and **7** (90.5 % from **5**), respectively. Recrystallization of the mixture of **7** and **9**, and **8** and **9** from hexane, followed by HPLC separation of these filtrates, gave **7** (11.1 % from **2**) and **9** (77.8 % from **2**), and **8** (14.5 % from **4**) and **9** (81.1 % from **4**), respectively. The ^{13}C and ^{1}H NMR spectra of **7**, **8** and **9** were superimposable on those of the known 2*R**, 3*R**, 6*R**, 7*R**, 9*S**, 10*S**-, 2*S**, 3*S**, 6*S**, 7*S**, 9*S**, 10*S**- and 2*S**, 3*S**, 6*R**, 7*R**, 9*S**, 10*S**-humulene 2,3;6,7;9,10-triepoxides,[3] and therefore, these configurations were depicted as **7**, **8** and **9** in Scheme 3. Although the HRMS of **6** showed the same molecular formula, $C_{15}H_{24}O_3$, as those of **7**, **8** and **9**, the triepoxide (**6**) differed from **7**, **8** and **9** in these ^{13}C and ^{1}H NMR spectra. In order to determine the configuration of **6**, a single crystal of **6** was used for X-Ray crystallography, which revealed its configuration, 2*R**, 3*R**, 6*S**, 7*S**, 9*S**, 10*S**-humulene 2,3;6,7;9,10-triepoxide, as depicted in Figure 5. The fractional atomic coordinates and the bond lengths and angles of **6** are listed in Tables 1 and 2.

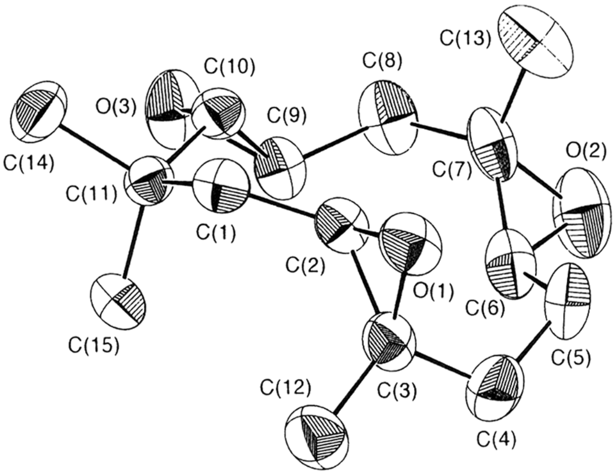


Figure 5. Perspective view of **6**. Hydrogen atoms were omitted.

The existence of the two stereoisomeric triepoxides (**7** and **9**, **6** and **8**, **8** and **9**, and **6** and **7**) derived from the pure diepoxide (**2**, **3**, **4**, and **5**) by the epoxidation reaction experimentally suggests that **2**, **3**, **4**, and **5** are in equilibrium between the main conformation (CT, CC, CT and TC) and the minor conformation (TC, TT, CC and TT) by the rotation of the 2,3- or 6,7-double bond planes, respectively (Scheme 4).

Consequently, the humulene 9,10-epoxide (**1**) in the complete epoxidation gave a mixture (97 % yield) of **6**, **7**, **8** and **9** in the ratio of **6** : **7** : **8** : **9** = 1.3 : 11.5 : 22.6 : 64.6, through the main and minor conformations of the 6,7;9,10- (**2** and **3**) and 2,3;9,10-diepoxides (**4** and **5**). It is thought that **6** is produced from the CC and TC and/or TT conformation of **1** through the minor TT conformation of **3** and **5** (Scheme 4). From the results of the conformational studies in the humulene diepoxides (**2**, **3**, **4** and **5**) and the configurational studies in the four humulene triepoxides (**6**, **7**, **8** and **9**), it is suggested that the 2,3- and 6,7-double bond planes in the original humulene 9,10-epoxide (**1**) are rotating as well as those in the diepoxides (**2**, **3**, **4**, and **5**). Therefore, it is postulated that the coupling pattern of the hydrogen at the 2 and 6 positions observed as ddq and tq in the ^{1}H NMR spectrum (CD$_3$COCD$_3$, 24.1 ˚C) of **1** is the average of the four possible conformations of **1**. Because rotation restriction of the double bond planes in **1** causes the change in the coupling pattern, we tried to observe the spectrum of **1** by lowering the temperature (-30, -40, -50 and -60 ˚C, see in EXPERIMENTAL), but could not observe any change in the coupling pattern, expect for the broadening of all peaks. Therefore, the population of the four possible conformations in **1** was calculated using Conflex with MM2 (extensive mode)[4] to give a ratio[5] of TT : TC : CC : CT = 2.9 : 34.7 : 18.6 : 42.1. Assuming that the diepoxides are produced in the calculated ratio from **1**, the product ratio (A) of the diepoxides was calculated as **2** (CT+TC) : **3** (CC+TT) = 76.8 : 21.5 and **4** (CT+CC) : **5** (TC+TT) = 60.7 : 37.6 by adding the population of the main and minor conformations. The conformational population (B) of these diepoxides was also calculated by Conflex with MM2 to give the ratio of CT : TC = 63.4 : 36.3 in **2**, CC : TT = 89.6 : 10.3 in **3**, CT : CC = 87.5 : 12.5 in **4** and TC : TT = 90.3 : 9.7 in **5**. Multiplying the reaction ratio (C) of the 6,7-position : 2,3-position = 59 : 41 experimentally obtained

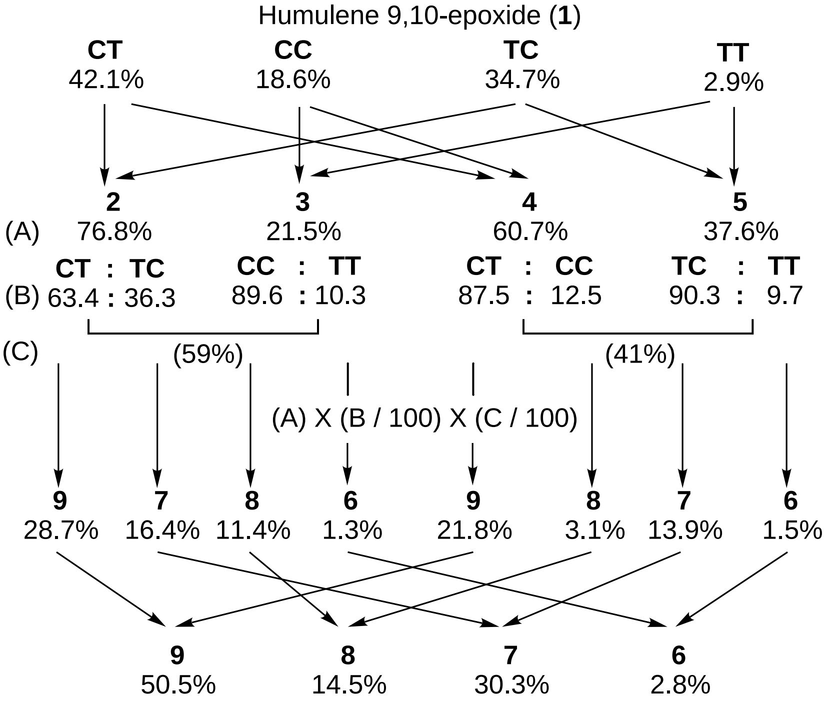


Figure 6. The calculated product ratio of the four triepoxides.

in the epoxidation reaction of **1** by the above calculated product ratio (A) and conformational population (B) of the diepoxides, the product ratio of the four triepoxides (**6**, **7**, **8** and **9**) that originated from the four possible conformations (TT, TC, CC and CT) of **1** is expected to be **6** : **7** : **8** : **9** = 2.8 : 30.3 : 14.5 : 50.5 (Figure 6). The calculated value is close to the experimental one (**6** : **7** : **8** : **9** = 1.3 : 11.5 : 22.6 : 64.6) obtained in this study. The result of the calculation by Conflex with MM2 (extensive mode) supports an equilibrium between the four possible conformations (TT, TC, CC and CT) in **1** (Scheme 3) and between the main and minor conformations in **2**, **3**, **4** and **5** (Scheme 4). This result also allows us to consider the rotation of the 2,3- and 6,7-double bond planes of **1** at low temperatures during the measurement of the ^{1}H NMR spectrum.

Thus, the epoxidation of the 6,7- and/or 2,3-double bond in **1** produced the four diepoxides (**2**, **3**, **4** and **5**) the main conformations of which were determined as CT, CC, CT and TC by X-Ray crystallography and NMR spectroscopy, respectively. During the epoxidation of the residual double bond in **3** and **5**, the new triepoxide (**6**) was first isolated as the minor product and the configuration was determined as 2*R**, 3*R**, 6*S**, 7*S**, 9*S**, 10*S** by X-Ray crystallography. Consequently, the humulene 2,3;6,7;9,10-triepoxide (**6**) was formed *via* the less stable TT conformation of **3** and **5** from **1** during the epoxidation reaction. No compound that originated from the TT conformation, expcet for the triepoxide (**6**), has been experimentally isolated and confirmed in humulene, the humulene mono-, di- and triepoxides, transannular cyclized compounds of humulene and the monoepoxides and cyclohumulanoids.[6]

Table 1. Fractional Atomic Coordinates and Equivalent Isotropic Thermal Parameters with Esd's in Parentheses for the Non-Hydrogen Atoms of Compounds (**3**), (**4**) and (**6**).

Compound **3**					Compound **4**				
Atom	x/a	y/b	z/c	U(iso)	Atom	x/a	y/b	z/c	U(iso)
O(17)	0.1513(3)	0.4601(1)	0.1100(3)	0.072(2)	O(17)	0.00509(9)	0.31888(13)	1.39898(29)	0.099(1)
O(16)	0.4440(3)	0.2580(2)	-0.0825(3)	0.079(2)	O(16)	0.21425(9)	0.19939(15)	0.70813(28)	0.097(1)
C(10)	0.1243(5)	0.4526(2)	-0.0553(4)	0.048(2)	C(2)	0.16563(12)	0.23100(17)	0.89138(36)	0.069(1)
C(9)	0.1718(5)	0.3936(2)	0.0368(4)	0.059(3)	C(7)	0.16330(12)	0.47198(16)	1.16162(36)	0.067(1)
C(8)	0.3334(5)	0.3646(2)	0.0324(5)	0.067(3)	C(11)	0.05272(12)	0.18489(18)	1.14761(39)	0.073(1)
C(4)	0.1082(5)	0.2448(2)	-0.3699(5)	0.061(3)	C(9)	0.05124(12)	0.37077(18)	1.23760(35)	0.069(1)
C(1)	-0.0509(5)	0.4320(2)	-0.2752(4)	0.054(3)	C(3)	0.24929(12)	0.25431(18)	0.89631(37)	0.070(1)
C(11)	-0.0385(5)	0.4640(2)	-0.1104(5)	0.052(3)	C(10)	0.07095(12)	0.27109(16)	1.29174(35)	0.068(1)
C(7)	0.3735(5)	0.3243(2)	-0.1120(5)	0.052(3)	C(6)	0.23029(13)	0.42434(18)	1.18350(37)	0.075(1)
C(6)	0.2903(4)	0.2610(2)	-0.1467(5)	0.053(2)	C(4)	0.27437(12)	0.35186(21)	0.82012(38)	0.082(1)
C(2)	-0.0146(5)	0.3544(2)	-0.2853[5)	0.053(3)	C(5)	0.29345(13)	0.42077(20)	1.01445(47)	0.088(2)
C(3)	0.0743(4)	0.3217(2)	-0.3883(5)	0.047(2)	C(8)	0.10003(13)	0.45278(17)	1.32655(40)	0.078(1)
C(5)	0.2697(5)	0.2308(2)	-0.3058(5)	0.059(3)	C(1)	0.12795(14)	0.15409(17)	1.03163(43)	0.081(1)
C(13)	0.4476(5)	0.3681(2)	-0.2329(6)	0.080(3)	C(13)	0.14393(14)	0.53878(20)	0.97110(45)	0.090(2)
C(14)	-0.1560(5)	0.4329(2)	-0.0022(5)	0.072(3)	C(14)	-0.01184(13)	0.20260(20)	0.97056(47)	0.091(2)
C(15)	-0.0685(5)	0.5432(2)	-0.1202(5)	0.075(3)	C(12)	0.30478(13)	0.20011(21)	1.04423(43)	0.091(2)
C(12)	0.1452(6)	0.3578(3)	-0.5256(5)	0.077(4)	C(15)	0.02776(17)	0.10281(20)	1.30632(55)	0.107(2)

Compound **6**									
Atom	x/a	y/b	z/c	U(iso)	Atom	x/a	y/b	z/c	U(iso)
O(1)	0.98511(8)	0.23943(13)	1.01429(8)	0.0591(6)	C(7)	0.70134(8)	0.46909(21)	0.96495(9)	0.0533(7)
O(2)	0.62261(8)	0.36380(19)	0.92365(10)	0.0791(7)	C(8)	0.68769(9)	0.58425(21)	1.05747(11)	0.0586(7)
O(3)	0.79364(8)	0.68740(14)	1.23570(9)	0.0653(6)	C(3)	0.92652(10)	0.16437(17)	1.08495(10)	0.0491(6)
C(1)	1.00112(8)	0.43856(16)	1.16999(10)	0.0453(6)	C(4)	0.83613(12)	0.08270(20)	1.02221(13)	0.0640(8)
C(11)	0.94750(8)	0.52695(14)	1.25032(9)	0.0387(5)	C(13)	0.74398(12)	0.54503(26)	0.87523(13)	0.075(1)
C(10)	0.86116(9)	0.61170(15)	1.17873(10)	0.0438(5)	C(6)	0.71069(10)	0.29889(21)	0.99001(11)	0.0569(7)
C(9)	0.76185(8)	0.55471(17)	1.16301(9)	0.0459(6)	C(14)	1.01557(11)	0.65436(18)	1.31465(14)	0.0603(7)
C(2)	0.93723(8)	0.33868(16)	1.08407(9)	0.0427(6)	C(5)	0.76654(11)	0.18039(22)	0.93680(12)	0.0637(8)
C(15)	0.91825(9)	0.41264(17)	1.33251(10)	0.0490(6)	C(12)	0.98290(15)	0.06372(20)	1.17692(13)	0.0717(9)

Table 2. Bond Lengths (Å) and Angles (deg.) of Compounds (**3**), (**4**) and (**6**).

	3	**4**	**6**		**3**	**4**	**6**
O(17)-C(10)	1.451(5)	1.471(3)		C(4)-C(3)	1.500(6)	1.496(4]	1.512(3)
O(16)-C(7)	1.427(5)			C(1)-C(11)	1.550(6)	1.540(4)	1.552(2)
O(17)-C(9)	1.423(5)	1.445(3)		C(11)-C(14)	1.509(7)	1.533(4)	1.543(2)
O(16)-C(6)	1.456(5)			C(7)-C(6)	1.438(6)	1.338(4)	1.466(3)
O(16)-C(2)		1.448(3)		C(6)-C(5)	1.498(6)	1.492(4)	1.503(3)
O(16)-C(3)		1.468(3)		C(3)-C(12)	1.502(6)	1.489(4)	1.498(3)
O(1)-C(2)			1.459(2)	C(10)-C(11)	1.518(6)	1.499(4)	1.523(2)
O(2)-C(7)			1.430(2)	C(8)-C(7)	1.503(6)	1.500(4)	1.533(3)
O(3)-C(10)			1.444(2)	C(4)-C(5)	1.541(6)	1.524(4)	1.519(3)
O(1)-C(3)			1.461(2)	C(1)-C(2)	1.511(6)	1.507(4)	1.494(2)
O(2)-C(6)			1.445(2)	C(11)-C(15)	1.529(5)	1.542(4)	1.511(2)
O(3)-C(9)			1.440(2)	C(7)-C(13)	1.483(7)	1.490(4)	1.502(3)
C(10)-C(9)	1.435(6)	1.461(4)	1.459(2)	C(2)-C(3)	1.335(6)	1.482(3)	1.476(2)
C(9)-C(8)	1.519(7)	1.507(4)	1.504(2)				

	3	**4**	**6**		**3**	**4**	**6**
C(2)-O(1)-C(3)			60.7(1)	C(11)-C(1)-C(2)	115.0(4)	115.0(2)	114.3(1)
C(10)-O(3)-C(9)			60.8(1)	C(1)-C(11)-C(15)	108.9(4)	108.1(3)	110.8(2)
O(3)-C(10)-C(11)			117.5(1)	C(10)-C(11)-C(15)	108.6(4)	107.9(2)	112.5(1)
O(3)-C(9)-C(8)			118.3(2)	C(15)-C(11)-C(14)	107.6(4)	108.8(2)	109.4(2)
O(1)-C(2)-C(1)			116.6(1)	C(10)-C(9)-C(8)	122.7(4)	121.0(2)	122.5(2)
O(2)-C(7)-C(13)			114.1(2)	C(8)-C(7)-C(6)	118.7(4)	119.3(3)	118.9(2)
O(1)-C(3)-C(2)			59.6(1)	C(9)-C(8)-C(7)	115.1(4)	108.6(2)	110.9(2)
O(1)-C(3)-C(12)			114.2(2)	C(2)-C(3)-C(4)	119.9(4)	118.7(2)	121.7(2)
O(2)-C(6)-C(5)			118.5(2)	C(4)-C(3)-C(12)	116.6(4)	116.6(2)	112.6(2)
C(7)-O(2)-C(6)			61.3(2)	C(7)-C(6)-C(5)	124.9(4)	126.5(3)	126.2(2)

O(3)-C(10)-C(9)			59.5(1)	C(10)-O(17)-C(9)	59.9(3)	60.2(2)
O(3)-C(9)-C(10)			59.7(1)	O(17)-C(10)-C(9)	59.1(3)	59.1(2)
O(1)-C(2)-C(3)			59.7(1)	O(17)-C(9)-C(8)	116.8(4)	117.5(2)
O(2)-C(7)-C(8)			116.3(2)	O(16)-C(7)-C(6)	61.1(3)	
O(2)-C(7)-C(6)			59.8(2)	O(16)-C(6)-C(5)	116.4(4)	
O(1)-C(3)- C(4)			114.9(2)	C(7)-O(16)-C(6)	59.8(3)	
O(2)-C(6)-C(7)			58.9(1)	O(17)-C(10)-C(11)	116.5(4)	116.6(2)
C(1)-C(11)-C(10)	107.2(4)	107.6(2)	107.4(1)	O(17)-C(9)-C(10)	61.0(3)	60.8(2)
C(1)-C(11)-C(14)	111.4(4)	110.7(2)	108.7(1)	O(16)-C(7)-C(8)	113.8(4)	
C(10)-C(11)-C(14)	113.0(4)	113.6(3)	107.9(2)	O(16)-C(7)-C(13)	115.6(4)	
C(11)-C(10)-C(9)	123.8(4)	126.1(2)	123.8(2)	O(16)-C(6)-C(7)	59.1(3)	
C(1)-C(2)-C(3)	128.0(4)	125.2(2)	127.1(2)	O(16)-C(2)-C(3)		60.1(2)
C(8)-C(7)-C(13)	113.5(4)	116.2(2)	113.7(2)	O(16)-C(3)-C(4)		111.3(2)
C(13)-C(7)-C(6)	123.0(4)	124.3(3)	122.5(2)	C(2)-O(16)-C(3)		61.1(2)
C(2)-C(3)-C(12)	123.5(4)	121.3(3)	121.9(2)	O(16)-C(2)-C(1)		117.1(2)
C(3)-C(4)-C(5)	112.8(4)	113.9(2)	117.4(2)	O(16)-C(3)-C(2)		58.8(2)
C(4)-C(5)-C(6)	111.9(4)	111.8(2)	112.6(2)	O(16)-C(3)-C(12)		115.5(3)

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. The NMR spectra were measured using JEOL JNN-EX 270 (270 MHz for ^{1}H, 67.5 MHz for ^{13}C) and 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) FT-NMR spectrometers. Chemical shifts are reported in δ units relative to internal Me_4Si. TLC: precoated TLC plated, silica gel 60 F-254 (Merck). Adsorption chromatography: silica gel 60, 70-230 mesh ASTM (Merck). HPLC: model 6000A solvent delivery system (Waters), differential refractometer R 401 (Waters).

Epoxidation reaction of humulene 9,10-epoxide (**1**): To a stirred solution of **1** (7.532 g, 34.24 mmol) in dry dichloromethane (100 mL) under an argon atmosphere at 0 ℃ was added 7.033 g (40.76 mmol) of *m*-CPBA (minimum 70%). After stirring for 2.5 h at 0 ℃ and then 15.5 h at rt, the epoxidation was complete. To the reaction mixture were added 122 mL of 1 M sodium hydroxide solution and 122 mL of 10 % (w / v) sodium thiosulfate solution, and then the mixture was extracted three times with dichloromethane. The extracts were washed with 1 M sodium hydroxide solution and water, and dried over anhydrous magnesium sulfate. Removal of the solvent yielded an oily mixture, of which the separation by silica gel column chromatography (ethyl acetate / hexane = 1 / 9, v / v) gave 7.856 g (97%) of a mixture of humulene 6,7;9,10- (**2** and **3**) and the 2,3;9,10-diepoxides (**4** and **5**). The ratio of (**2**+**3**) : (**4**+**5**) = 59 : 41 was calculated from the HPLC peak areas under the following separation conditions. Although it is possible that the epoxidation reaction of **1** gives the triepoxides as a minor product, we did not investigate it further.

Separation of the humulene diepoxide mixture into humulene 6,7;9,10- (**2**+**3**) and 2,3;9,10-diepoxides

(**4**+**5**): A 50% ethanol solution (v / v) of the above mixture (7.856 g) was separated by HPLC using a 10 x 244 mm column of RP-18 (7 μm, Merck, H_2O / EtOH = 3 / 7, rate of flow = 1.2 mL / min) into an oily mixture of humulene 6,7;9,10-diepoxides (second peak, **2**+**3**, 3.91 g, 48.4% from **1**) and a crystalline mixture of humulene 2,3;9,10-diepoxides (first peak, **4**+**5**, 2.71 g, 33.6% from **1**). The ratio of **2** : **3** = 69 : 31 and **4** : **5** = 85 : 15 was calculated from the HPLC peak areas under the following separation condition.

Separation of humulene 6,7;9,10- (**2**+**3**) and 2,3;9,10-diepoxides (**4**+**5**) into **2** and **3**, and **4** and **5**, respectively: A 50% hexane solution (v/v) of a mixture (3.91 g) of the humulene 6,7;9,10-diepoxides (**2**+**3**) was separated by HPLC using two 10 x 250 mm columns of Inertsil SIL 100-5 (GL Science, Inc., EtOAc / hexane = 1 / 9, rate of flow = 4.0 mL / min) into **2** (first peak, oil, 2.48 g, 30.7% from **1**) and **3** (second peak, crystal, 1.16 g, 14.4% from **1**). Separation of the mixture (2.71 g) of the humulene 2,3;9,10-diepoxides (**4**+**5**) was carried out in the same manner as described above to give **4** (first peak, crystal, 2.12 g, 26.3% from **1**) and **5** (second peak, crystal, 0.372 g, 4.6% from **1**).

2: oil; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 0.64 (1H, dd, *J*=10.3, 12.9 Hz, 8βH), 0.78, 1.10, 1.39, 1.69 (each 3H, s), 1.35 (1H, m, 5H), 1.96 (1H, br d, *J*=14.9 Hz, 1αH), 2.16 (1H, dd, *J*=7.3, 13.3 Hz, 4αH), 2.22 (1H, d, *J*=3 Hz, 10H), 2.24 (1H, dd, *J*=10.6, 14.9 Hz, 1βH), 2.26 (1H, m, 4βH), 2.30 (1H, m, 5H), 2.48 (1H, dd, *J*=4.6, 10.3 Hz, 6H), 2.68 (1H, dd, *J*=3, 12.9 Hz, 8αH), 2.80 (1H, dt, *J*=3, 10.3 Hz, 9H), 5.20 (1H, br d, *J*=10.6 Hz, 2H) ppm; ^{13}C NMR ($CDCl_3$, 400 MHz) δ 15.2 (q, 3Me), 17.3 (q), 19.3 (q), 24.8 (t, 5C), 28.5 (q), 34.1 (s, 11C), 36.2 (t, 4C), 39.1 (t, 1C), 43.6 (t, 8C), 53.0 (d, 9C), 57.8 (s, 7C), 61.7 (d, 6C), 66.1 (d, 10C), 122.8 (d, 2C), 133.7 (c, 3C) ppm; HRMS (EI): m/z 236.1752 (M^+, $C_{15}H_{24}O_2$ requires 236.1777).

3: mp 69-71 ˚C (hexande); ^{1}H NMR ($CDCl_3$, 400 MHz), δ 0.77, 1.13, 1.20 (each 3H, s), 1.44 (1H, dddd, *J*=2.4, 2,4, 7.2, 14.5 Hz, 5H), 1.54 (1H, dd, *J*=8.3, 13.7 Hz, 8H), 1.61 (3H, br s, 3Me), 1.92 (1H, br d, *J*=15.0 Hz, 1H), 2.00 (1H, ddd, *J*=2.4, 7.2, 14.5 Hz, 5H), 2.16 (1H, dd, *J*=5.5, 13.7 Hz, 8H), 2.22 (2H, dd, *J*=2.4, 7.2 Hz, $4H_2$), 2.39 (1H, dd, *J*=11.0, 15.0 Hz, 1H), 2.48 (1H, d, *J*=2.7 Hz, 10H), 2.80 (1H, d, *J*=2.4 Hz, 6H), 2.82 (1H, ddd, *J*=2.7, 5.5, 8.3 Hz, 9H), 5.15 (1H, br d, *J*=11.0 Hz, 2H) ppm; ^{13}C NMR ($CDCl_3$, 270 MHz) δ 16.1 (q, 3Me), 18.0 (q), 20.3 (q), 25.0 (t, 5C), 29.5 (q), 34.4 (s, 11C), 36.6 (t, 4C), 38.8 (t, 1C), 41.9 (t, 8C), 51.7 (d, 9C), 58.6 (s, 7C), 61.4 (d, 6C), 63.2 (d, 10C), 122.9 (d, 2C), 133.7 (s, 3C) ppm; HRMS (EI): m/z 236.1752 (M^+, $C_{15}H_{24}O_2$ requires 236.1777).

4: mp 66-69 ˚C (hexande); ^{1}H NMR ($CDCl_3$, 270 MHz), δ 0.84, 1.09, 1.25, 1.71 (each 3H, s), 1.15

(1H, dd, *J*=14.0, 15.0 Hz, 4βH), 1.57 (1H, dd, *J*=7.6, 15.2 Hz, 1βH), 1.63 (1H, dd, *J*=9.9, 12.9 Hz, 8βH), 1.68 (1H, dd, *J*=1.3, 15.2 Hz, 1αH), 2.07 (1H, ddd, *J*=5.2, 7.9, 13.6 Hz, 5βH), 2.12 (1H, ddd, *J*=1.8, 5.2, 15.0 Hz, 4αH), 2.30 (1H, dddd, *J*=1.8, 7.9, 13.6, 14.0 Hz, 5αH), 2.36 (1H, d, *J*=2.3 Hz, 10H), 2.58 (1H, dd, *J*=1.3, 7.6 Hz, 2H), 2.68 (1H, dd, *J*=3.6, 12.9 Hz, 8αH), 3.01 (1H, ddd, *J*=2.3, 3.6, 9.9 Hz, 9H), 5.28 (1H, t, *J*=7.9 Hz, 6H) ppm; ^{13}C NMR ($CDCl_3$, 270 MHz) δ 16.4 (q), 17.79 (q), 18.2 (q), 23.4 (t, 5C), 29.0 (q), 33.2 (s), 38.3 (t, 4C), 38.7 (t, 1C), 41.5 (t, 8C), 56.5 (d, 9C), 61.0 (s), 61.6 (d, 2C), 65.4 (d, 10C), 125.5 (d, 6C), 132.6 (s, 7C) ppm; HRMS (EI): m/z 236.1766 (M^+, $C_{15}H_{24}O_2$ requires 236.1777).

5: mp 85-87 °C (hexande); ^{1}H NMR ($CDCl_3$, 270 MHz), δ 0.71, 1.14, 1.30, 1.77 (each 3H, s), 1.18 (1H, dd, *J*=4.7, 12.8 Hz, 4βH), 1.49 (1H, dd, *J*=5.2, 15.8 Hz, 1αH), 1.53 (1H, dd, *J*=3.0, 15.8 Hz, 1βH), 1.63 (1H, dd, *J*=9.9, 12.0 Hz, 8βH), 2.12 (1H, dd, *J*=3.3, 12.8 Hz, 5βH), 2.17 (1H, dt, *J*=12.8, 3.3 Hz, 4αH), 2.32 (1H, dddd, *J*=3.3, 4.7, 11.9, 12.8 Hz, 5αH), 2.66 (1H, dd, *J*=3.3, 12.0 Hz, 8αH), 2.67 (1H, d, *J*=2.7 Hz, 10H), 2.79 (1H, dd, *J*=3.0, 5.2 Hz, 2H), 2.90 (1H, ddd, *J*=2.7, 3.3, 9.9 Hz, 9H), 5.10 (1H, br d, *J*=11.9 Hz, 6H) ppm; ^{13}C NMR ($CDCl_3$, 270 MHz) δ 17.0 (q), 17.7 (q, 7Me), 22.9 (q), 24.6 (t, 5C), 26.8 (q), 32.5 (s, 11C), 38.4 (t, 4C), 40.9 (t, 1C), 43.3 (t, 8C), 54.8 (d, 9C), 60.2 (s, 3C), 60.4 (d, 2C), 64.7 (d, 10C), 127.9 (d, 6C), 131.2 (s, 7C) ppm; HRMS (EI): m/z 236.1781 (M^+, $C_{15}H_{24}O_2$ requires 236.1777).

Epoxidation of the above pure humulene diepoxides (**2**, **3**, **4** and **5**): Epoxidation of **2** (1.0 g, 4.237 mmol) and **4** (1.0 g, 4.237 mmol) with 0.877 g (5.084 mmol) of *m*-CPBA (minimum 70%) in dry dichloromethane (15 mL) under an argon atmosphere at 0 °C for 4 h, extractive workup and chromatographic separation (silica gel, EtOAc / hexane = 3 / 17) were carried out in the same manner described above to give a crystalline mixture (1.07 g, 100%, ratio of **7** : **9** = 14 : 86) of **7** and **9**, and a crystalline mixture (1.07 g, 100%, ratio of **8** : **9** = 15 : 85) of **8** and **9**, respectively. Epoxidation of **3** (1.16 g, 4.915 mmol) and **5** (0.372 g, 1.576 mmol) in dry dichloromethane (15 and 5 mL) with *m*-CPBA {1.01 g (5.853 mmol) and 0.324 g (1.877 mmol)} and then chromatographic separation were also achieved by the same method as shown in **2** and **4** to give a crystalline mixture (1.24 g, 100%, ratio of **6** : **8** = 5 : 95) of **6** and **8**, and a crystalline mixture (0.397 g, 100%, ratio of **6** : **7** = 6 : 94) of **6** and **7**, respectively. The ratio of these mixtures was calculated from the HPLC peak areas under the following separation conditions.

Separation of four mixtures of humulene triepoxides produced from **2**, **3**, **4** and **5** into **6**, **7**, **8** and **9**: Two mixtures of the epoxidation products of **2** and **4**, {**7**+**9** (1.07 g)} and {**8**+**9** (1.07 g)}, were recrystallized from hexane to give **9** (0.629 g and 0.747 g) and two filtrates, which were respectively separated into **7** (second peak, 0.119 g, 11.1% from **2**) and **9** (first peak, 0.202 g), and **8** (second peak, 0.155 g, 14.5% from **4**) and **9** (first peak, 0.119 g) by HPLC using two 10 x 250 mm columns of Inertsil SIL 100-5 (GL Science, Inc., EtOAc / hexane = 1 / 3, rate of flow = 4.0 mL / min). The total yield of **9** from **2** and **4** was 77.8% and 81.1%. The residual two mixtures, **6**+**8** (1.24 g) and **6**+**7** (0.397 g) were also separated by fractional crystallization from benzene and the HPLC separation of these filtrates to give **6** (first peak, 58 mg, 4.7% from **3**) and **8** (second peak, 1.162 g, 93.8% from **3**), and **6** (first peak, 19 mg, 4.8% from **5**) and **7** (second peak, 0.359 g, 90.5% from **5**), respectively. The ^{1}H and ^{13}C NMR spectra of **7**, **8** and **9** in $CDCl_3$ were superimposable on the authentic samples of the (2*R**, 3*R**, 6*R**, 7*R**, 9*S**, 10*S**)-, (2*R**, 3*R**, 6*R**, 7*R**, 9*R**, 10*R**)- and (2*S**, 3*S**, 6*R**, 7*R**, 9*S**, 10*S**)-humulene 2,3;6,7;9,10-triepoxides.^{3}

6: mp 147-149 ℃ {20 % (v/v) EtOAc / hexane}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.72, 1.15, 1.32, 1.35 (each 3H, s), 1.37 (1H, m, 5H), 1.43 (1H, dd, *J*=11.0, 14.2 Hz, 1H), 1.49 (1H, dd, *J*=9.6, 14.0 Hz, 8H), 1.83 (1H, m, 5H), 1.85 (1H, m, 4H), 2.01 (1H, m, 4H), 2.07 (1H, dd, *J*=3.1, 14.2 Hz, 1H), 2.35 (1H, dd, *J*=4.3, 14.0 Hz, 8H), 2.72 (1H, d, *J*=2.5 Hz, 10H), 2.77 (1H, ddd, *J*=2.5, 4.3, 9.6 Hz, 9H), 2.92 (1H, dd, *J*=3.1, 11.0 Hz, 2H), 2.94 (1H, m, 6H) ppm; ^{13}C NMR ($CDCl_3$, 400 MHz) δ 18.3 (q), 20.2 (q), 20.9 (q), 21.0 (t, 5C), 28.3 (q), 32.7 (s, 11C), 34.3 (t, 4C), 38.5 (t, 8C), 39.9 (t, 1C), 53.2 (d, 9C), 58.8 (s), 59.2 (d, 2 or 6C), 60.1 (s), 61.7 (d, 6 or 2C), 63.8 (d, 10C) ppm; HRMS (EI): m/z 252.1722 (M^+, $C_{15}H_{24}O_3$: requires 252.1726). *Anal.* Calcd for $C_{15}H_{24}O_3$: C 71.39; H 9.59. Found: C 71.29, H 9.57.

X-Ray crystallography of **3**, **4**, and **6**: The X-Ray crystallography of the single crystals of **3**, **4** and **6** obtained by recrystallization from hexane, ethanol and 20 % (v/v) EtOAc / hexane was carried out on a MAC Science MXC3k four-circle diffractometer with graphite-monochromatized MoKα radiation (λ=0.71073 Å) using a ω-2θ scan technique. A total of 1523, 2322 and 3281 independent reflections was collected for compounds (**3**), (**4**) and (**6**), respectively, and these structures were solved by direct methods. Crystal data **3**: $C_{15}H_{24}O_2$, F.W.=236.35, orthorhombic, space group $P2_12_12_1$, a = 8.751(2) Å, b = 19.010(4) Å, c =8.621(2) Å, V = 1434.1(6) Å^3, Z = 4, D_{calc} = 1.095 g/cm^3, μ(MoKα) = 0.658 cm^{-1}, R = 0.055, Rw = 0.059, 680 observed reflections [$I \geq 3\sigma(I)$] used in the refinement. Crystal data **4**: $C_{15}H_{24}O_2$, F.W.=236.40, monoclinic, space group $P2_1/n$, a = 17.286(2) Å, b = 13.907(2) Å, c =

5.8893(9) Å, β = 91.03(1)°, V = 1415.5(3) Å^3, Z = 4, D_{calc} = 1.109 g/cm^3, μ(MoKα) = 0.667 cm^{-1}, R = 0.076, Rw = 0.081, 1212 observed reflections [$I \geq 3\sigma(I)$] used in the refinement. Crystal data **6**: $C_{15}H_{24}O_3$, F.W.=252.40, monoclinic, space group $P2_1/n$, a = 14.147(4) Å, b = 8.419(3) Å, c = 12.238(4) Å, β = 102.10(3)°, V = 1425.3(9) Å^3, Z = 4, D_{calc} = 1.176 g/cm^3, μ(MoK α) = 0.075 cm^{-1}, R = 0.060, Rw = 0.069, 1793 observed reflections [$I \geq 3\sigma(I)$] used in the refinement. The fractional atomic coordinates and the bond lengths and angles of **3**, **4** and **6** are listed in Tables 1 and 2.

Measurement of NMR spectrum of **1** at low temperature: The ^{1}H NMR spectrum (270 MHz) in CD_3COCD_3 of **1** was measured at 24.1, -30, -40, -50 and -60 ℃. Although all peaks in the spectrum measured at 24.1 ℃ were gradually broad with decreasing the temperature, these coupling patterns did not change without a slight change in the chemical shift. The spectral data of **1** at 24.1 and -60 ℃ are shown as follows.

Measurement at 24.1 ℃: δ 0.72 (3H, s, 11-Me), 1.03 (s, 3H, 11-Me), 1.44 (1H, dd, $J_{8\text{-}8}$ = 8.6, $J_{8\text{-}9}$ = 6.6 Hz, 8-H), 1.55 (3H, dd, $J_{3\mathrm{Me}\text{-}1}$ = $J_{3\mathrm{Me}\text{-}2}$ = 0.8 Hz, 3-Me), 1.65 (3H, d, $J_{7\mathrm{Me}\text{-}6}$ = 0.7 Hz, 7-Me), 1.87 (1H, ddq, $J_{1\text{-}2}$ = $J_{1\text{-}3\mathrm{Me}}$ = 0.8, $J_{1\text{-}1}$ = 9.8 Hz, 1-H), 2.04 - 2.20 (4H, m, 4- and 5-H_2), 2.27 (1H, d, $J_{10\text{-}9}$ = 1.5 Hz, 10-H), 2.28 (1H, dd, $J_{1\text{-}1}$ = 9.8, $J_{1\text{-}2}$ = 6.8 Hz, 1-H), 2.65 (1H, dd, $J_{8\text{-}8}$ = 8.6, $J_{8\text{-}9}$ = 2.8 Hz, 2-H), 2.93 (1H, ddd, $J_{9\text{-}10}$ = 1.5, $J_{9\text{-}8\mathrm{H}_2}$ = 2.8, 6.6 Hz, 9-H), 5.00 (1H, ddq, $J_{2\text{-}3\mathrm{Me}}$ = $J_{2\text{-}1}$ = 0.8, $J_{2\text{-}1}$ = 0.8, J $_{2\text{-}1}$ = 6.8 Hz, 2-H), 5.05 (1H, tq, $J_{6\text{-}5\mathrm{H}_2}$ = 5.0, $J_{6\text{-}7\mathrm{Me}}$ = 0.7 Hz, 6-H).

Measurement at -60 ℃: δ 0.72 (3H, br s, 11-Me), 1.01 (3H, br s, 11-Me), 1.45 (1H, br dd, J = 6.6, 8.6 Hz, 8-H), 1.56 (3H, br s, 3-Me), 1.68 (3H, br s, 7-Me), 1.85 (1H, br d, J = 9.8 Hz, 1-H), 2.00 - 2.20 (4H, br m, 4- and 5-H_2), 2.31 (1H, br d, J = 1.5 Hz, 10-H), 2.31 (1H, br dd, J = 6.8, 9.8 Hz, 1-H), 2.64 (1H, br dd, J = 2.8, 8.6 Hz, 8-H), 2.98 (1H, br ddd, J = 1.5, 2.8, 6.6 Hz, 9-H), 4.99 (1H, br d, J = 6.8 Hz, 2-H), 5.08 (1H, br t, J = 5.5 Hz, 6-H).

REFERENCES AND NOTES

1. H. Shirahama, E. Osawa, and T. Matsumoto, *J. Am. Chem. Soc.,* 1980, **102**, 3208; *Tetrahedron Lett.*, **1978**, 1987.

2. H. Shirahama, K. Hayano, Y. Kanemoto, S. Misumi, T. Ohtsuka, N. Hashiba, A. Furusaki, S. Murata, R. Noyori, and T. Matsumoto, *Tetrahedron Lett.*, 1980, **21**, 4835; H. Shirahama, K. Hayano, T. Ohtsuka, E, Osawa, and T. Matsumoto, *Chem. Lett.*, 1981, 351; K. Hayano and H. Shirahama, *Chem. Lett.*, 1993, 1151.

3. K. Hayano and K. Mochizuki, *Heterocycles*, 1997, **45**, 1573; K. Hayano, T. Shinjo, and K. Mochizuki, *Heterocycles*, 1996, **43**, 523.
4. H. Goto and E. Osawa, *J. Chem. Soc., Perkin Trans. 2*, 1993, 187.
5. Conflex (extensive mode) with MM2 in CAChe (windows version 3.2, Oxford Molecular, Sony Tektronix) was used.
6. K. Hayano, N. Ito, M. Sato, M. Takishima, and K. Mochizuki, *Heterocycles*, 1998, **48**, 1747 and references cited therein.