Tetrahedron Vol. 47, No. 6, pp. 933-940, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

HYDROLYSIS PRODUCTS OF HUMULENE DIEPOXIDE A

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(*Received in USA* 9 *October* 1990)

Abstract: Humulene diepoxide A, a flavoring compound found in hops, undergoes hydrolysis reactions upon boiling at pH 4. More than ten hydrolysis products were produced, of which five, 1–5 have been isolated and their structures determined. Two of these compounds, 1 and 2, are diastereomeric humuladiene triols, one is an exo–methylene diol 3, another is a saturated tricyclic triol 4, and the last is a bicyclic triol with an exo–methylene group 5. Aroma/flavor profiles of compounds 1 and 2 indicate that 2 would be more desirable than 1 in brewing.

INTRODUCTION

"Noble hop" aroma is an elusive flavor note imparted to beer by certain hop varieties. Older terminology referred to "kettle hop" aroma which was indicative of a certain aroma or flavor imparted only after vigorous boiling of "aroma hops" in the wort. Brewers and hop researchers do not agree entirely on the chemical or sensory nature of this flavor note, but it is commonly described as spicy or herbal.

The long boiling time is important, as most of the mass (80–90%) of typical hop oil consists of terpene and sesquiterpene hydrocarbons which are either steam distilled out of the wort, polymerized, or oxidized to water soluble compounds. The oxygenated sesquiterpenes rather than the hydrocarbons are considered to be the important aroma contributors.

There are two types of hops of commercial significance, bittering hops and aroma hops. High humulene concentrations have been associated with aroma hops. Humulene oxidation products, epoxides, alcohols and ketones may already be present in hop cones when harvested, but on storage under ambient conditions, the relative concentrations of these compounds increases[1]. One of the major humulene oxidation products present in hops whose concentration increases on storage is humulene diepoxide A. Recently, we synthesized this compound and determined its stereochemistry by X–ray crystallography[2]. We have now elucidated the structures of some of the products of hydrolysis of this compound and wish to report on these studies.

EXPERIMENTAL SECTION

NMR spectra were obtained for samples in perdeutero methanol or dimethyl sulfoxide. Chemical shifts (ppm) were referenced to the solvent line and reported as relative to tetramethylsilane.

Mass spectra were obtained with a Finnigan model 4023 quadrupole mass spectrometer using a 0.32 mm i.d. × 30 m DB–5 fused silica capillary column (J & W Scientific, Inc., Rancho Cordova, CA) for the trimethylsilylimidazole derivatized samples and a 0.32 mm i.d. × 30 m Supelcowax 10 fused silica capillary column (Supelco, Inc., Bellefonte, PA) for the hydrolysis products of the humulene diepoxides. Helium was used as the carrier gas at a flow rate of 25 cm/sec. The silylated samples were injected in the splitless mode. The oven temperature was programmed from 120 °C (held for one min) to 230 °C at 3 °C/min. The injector temperature was set at 250 °C and the transfer line at 275 °C. The ion source temperature was 140 °C; the electron energy was

70 eV. Mass spectra were taken every second, scanning from 40 to 600 amu. CI of purified samples were obtained in the positive ion (PI) mode, using ammonia as the reagent gas at a pressure of 0.6 Torr. Routine GC of samples were obtained with a Hewlett–Packard model 5830 GC equipped with a Hewlett–Packard 8850A GC terminal to determine the peak area and retention times. The split ratio was 50 and the columns and conditions wer the same as in GC–MS experiments.

Hydrolysis of Humulene Diepoxide A. Humulene diepoxide A was prepared and purified as described by Peacock and Deinzer[2]. A pH 4 solution of this compound (0.2 mg/mL) containing 3% ethanol was added to a rapidly vortexing solution of 10 mM sodium acetate buffer with pH 4 and boiled for 2 h. The reaction was quenched by cooling on ice and neutralizing with ammonium hydroxide. The reaction mixture was extracted by various methods including continuous liquid–liquid extraction for 24 h using dichloromethane. It was further purified by liquid chromatography as described by Lam et al.[1] and then freeze–dryed (aqueous pH 4 solution was used).

Isolation of Hydrolysis Products. The residue from the purified reaction mixture was dissolved in dichloromethane:methanol (7:1) (v/v) and eluted through a 2 × 25 cm silica gel (50 g of Lichoprep Si 60, E.M. Science) column that was equilibrated with dichloromethane:acetonitrile (4:1) (v/v). The flow rate was 10 mL/min and 20 mL fractions were collected. The solvent systems used were as follows: three 200 mL solutions of dichloromethane:acetonitrile (4:1), (2:1), and (1:1) in that order were employed followed by 200 mL dichloromethane:acetonitrile:methanol (49:49:2) and finally 300 mL of dichloromethane:acetonitrile:methanol (12:12:1). Fractions were monitored by thin layer chromatography and fractions with similar spots (stained with iodine) were pooled. The major (by mass) fractions were further purified by reverse–phase HPLC using a preparative 22 × 250 mm, 10 μ, octadecyl, Econosyl column (Altech Associates, Inc., Deer Field, IL). The eluant was monitored by absorbance at 210 nm. The solvent systems used were: methanol:water (43:57) for compound 1 (see Figure 1), methanol:water (1:1) for compound 2 and acetonitrile:water (1:4) for compounds 4 and 5. The organic solvents were removed by rotoevaporation and the aqueous solution was freeze–dried.

The compounds were derivatized with TMS imidazole:pyridine (2:1, v/v, both from Pierce Chemical Co., Rockford, IL) for 1.5 h at 85 °C. A kinetic study indicated that the reaction was completed within 1 h. The reacted samples were injected directly onto the GC column.

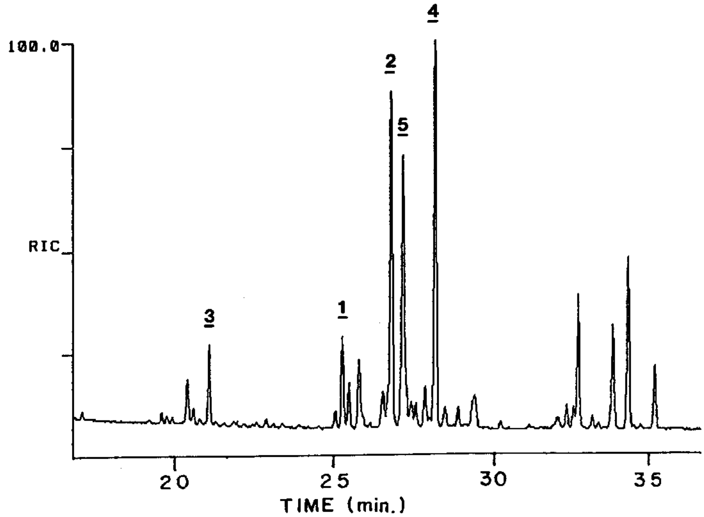


FIGURE 1. GC/MS chromatogram of trimethylsilyl ethers of hydrolysis products of humulene diepoxide A.

RESULTS AND DISCUSSION

When humulene diepoxide A is boiled in aqueous solution at pH 4 for two hours, a variety of hydrolysis products are obtained with less than 5% of the starting compound remaining. GC/MS analysis of the mixture of trimethylsilyl ether derivatives revealed there were three prominent peaks and more than ten lesser peaks (Figure 1). All of the major and most of the minor peaks were silylated as indicated by the relatively intense $(CH_3)_3Si^+$ ion peak with m/z 73[3]. Some GC/MS peaks had molecular ions with m/z 470, indicating that these compounds have three hydroxyl groups.

The EI mass spectra of compounds 1, 2, 4, and 5 had very small molecular ion peaks at m/z 254. The positive ion chemical ionization (PICI) mass spectra of purified compounds 1, 2, 4 and 5 with ammonia as reagent gas revealed that all of the compounds have prominent ion peaks with m/z 272 and 254. Ammonia PICI spectra of terpene alcohols[4] with a peak at mass 272 is due to $(M+NH_4)^+$ and the peak at mass 254 to $(M+NH_4-H_2O)^+$. Mass spectrometry of the TMS derivatives of compounds 1 and 2 show a low intensity molecular ion peak with m/z 470 which indicates that three silyl groups have been attached to each of the molecules. Moreover, the TMS derivatives of compounds 1, 2, and 4 each have a moderately intense peak with m/z 147 due to the $[(CH_3)_2SiOSi(CH_3)_3]^+$ suggesting that the TMS derivatives are in close proximity and able to react[5]. Thus, it is concluded that at least three vicinal diols are produced upon hydrolysis of one of the epoxide functionalities of humulene diepoxide A. In addition to a peak for $(CH_3)_3Si^+$, the spectrum of compound 5 showed a peak at m/z 131 due to $[(CH_3)_3SiOC(CH_3)_2]^+$. The highest masses for the silylated derivatives of compounds 4 and 5 were observed with m/z 365 and m/z 380, respectively. In summary, based largely on the PICI mass spectrometry results, it is concluded that compounds 1, 2, and 5 are unsaturated cyclic triols with two double bonds or unsaturated bicyclic triols with just one double bond.

With the help of the $^1H^1H$ and $^1H^{13}C$ correlation NMR spectra, structures have been proposed for compounds 1–5. The NMR spectra of compounds 1 and 2 (Table I) are similar with regard to

Table I. Carbon-13 and Proton NMR Data of Compounds 1 and 2

	^{13}C Shift[a] (ppm)		^{1}H Chemical Shift[a](ppm), J. (Hz)	
Carbon No.	1	2	1	2
1	36.71	36.73	--	--
2	51.38	52.15	1.46 (dd, J=2.7,14.1), 1.72 (dd, J=5.6,14.2)	1.51 (dd, J=3.0,14.1), 1.70 (dd, J=5.7)
3	67.90	67.71	4.54 (dd, J=2.5,5.6)	4.53 (dd, J=3.0,5.4)
4	138.89	139.52	--	--
5	125.01	124.40	5.27 (d,13.0)	5.28 (dd, J=1.7,11.9)
6	32.68	23.42	1.68 (m), 2.98 (dt)	1.58 (m), 2.69 (dt, J=13.0)
7	79.96	79.86	3.13 (dd, J=2.5,6.9)	3.19 (dd, J=1.1,7.3)
8	76.87	75.98	--	--
9	46.24	46.88	2.04 (dd, J=8.3,13.9), 2.32 (dd, J=5.9,13.9)	2.18 (dt, J=7.5,13.7), 2.29 (dt, J=4.6,13.6)
10	124.20	123.22	5.49 (m, J=8.6,6.2)	5.41 (b, 2H)
11	143.23	144.46	5.30 (d, J=16)	
12,13,15	24.88, 27.47, 30.84	20.69, 25.26, 30.18	0.97 (s, CH_3,3H) 1.16 (s, CH_3,3H), 1.23 (s, CH_3,3H)	0.98 (s, CH_3,3H) 1.15 (s, CH_3,6H)
14	17.54	17.58	1.69 (s, CH_3,3H)	1.70 (d, J=1.3, CH_3,3H)

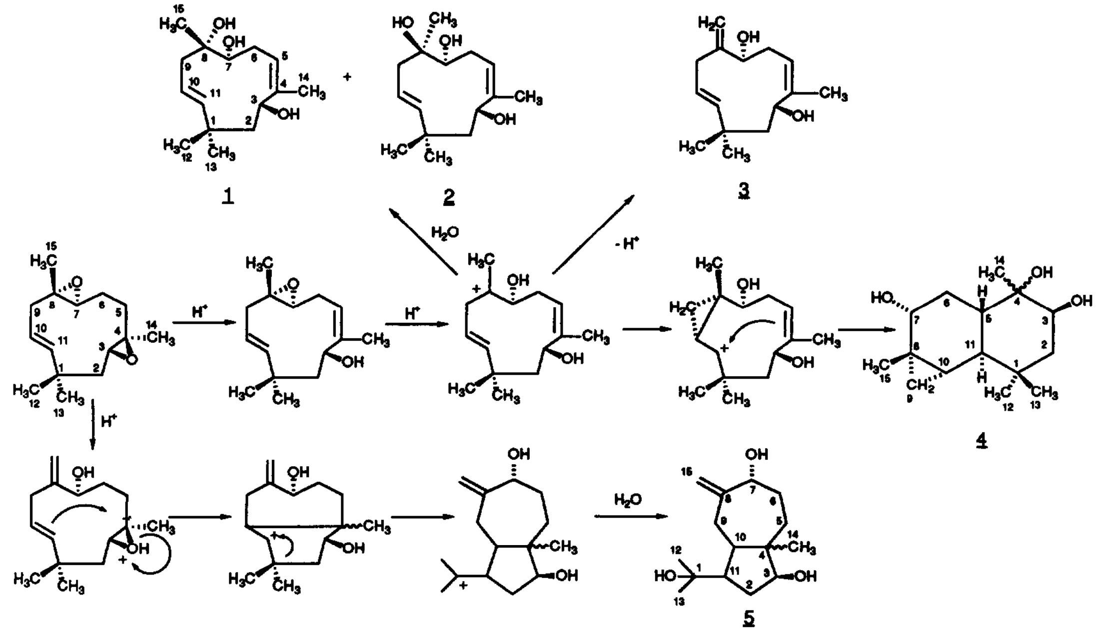
[a] Proton and carbon-13 NMR spectra were obtained in CD_3OD; b, s, d, dd, dt, m refers to broad, singlet, doublet, doublet of doublet, doublet of triplet, and multiplet, respectively.

chemical shift and $^1H^1H$ and $^1H^{13}C$ correlations. The only major difference in the NMR spectra of these two compounds is that compound 1 shows four resolved methyl groups, whereas compound 2 shows one methyl group resonance with twice the intensity of two others. In each case, there are four methyl groups and the compounds are believed to be stereoisomers.

If typical hydrolyses of the epoxide linkages take place under mildly acidic conditions, the reaction would proceed by protonation of the epoxide oxygen and ring opening in the direction that would yield the more stable carbonium ion. The reaction is probably intermediate between S_{N_1} and S_{N_2} as the ratio of compounds 1 and 2 suggest. The homoallylic ion involving the C10–C11 electrons could stabilize an intermediate carbonium ion. The reaction may involve an intermediate carbonium ion or an S_{N_2} displacement by water to form the vicinal diol. An elimination reaction to form an olefin also is involved. The Carbon–13 spectrum shows four downfield olefinic carbons in compounds 1 and 2. If it is assumed that the basic eleven–membered ring structure remains intact, then an elimination reaction would introduce a double bond, one of which would have to be substituted with a methyl group because of the absence of a proton as shown by the ^1H–^{13}C correlation spectrum. The chemical shifts, 138.89 ppm and 139.52 ppm for carbons substituted with methyl groups, i.e., C4 in 1 and 2 respectively, are in the correct range. The other epoxide ring would undergo displacement to give the vicinal diol and in both compounds 1 and 2 the carbons bearing the hydroxyl groups, i.e., C8, do not have protons. The chemical shifts, 76.87 ppm and 75.87 ppm, respectively, are correct for tertiary alcohols. The rest of the carbon–13 and proton assignments are straight forward.

The stereochemistry of the reactions should be predictable on the basis of commonly accepted mechanisms (Scheme I). If it is assumed that the epoxide ring opening reactions proceed in the direction of the more stable carbonium ions, the chirality at C3 and C7 should remain the same

SCHEME I



as in the starting compound. Anti–substitution at C8 would be preferred over *syn* substitution[6] and it is noted that about three times as much compound 2, i.e., the *trans*–diol, is formed. Hydrogen bonding between the hydroxyl groups give distinctly different chemical shifts. This would support the structural assignment given for compound 1. MMX calculations suggest the strain energy for compound 1 should be about 1.3 kcal lower than for compound 2.

From the mass spectrum of the TMS derivative of compound 3 it was concluded that there were two hydroxyl groups on the molecule. This is also clearly shown in the proton NMR spectrum by resonances at 3.91 ppm and 4.14 ppm (Table II). The proton spectrum shows three double bonds, one of which has protons with first order coupling only to each other. This clearly indicates an exomethylene group with the two protons on the same carbon atom. The structure of the compound is proposed to be that derived from elimination of the two epoxides. The stereochemistry should result from simple ring opening of the epoxide and chiriality at C3 and C7 should be retained. Namikawa and co–workers have shown that humulene–7,8–epoxide under mildly acid hydrolysis gives, among other compounds, humulenol II, which is the simple elimination reaction product with the stereochemistry of the starting compound retained[7].

In the mass spectra of compound 4 it was noted that the silyl ether derivative showed a peak at m/z 147, indicative of a vicinal diol. The proton NMR data clearly showed the presence of a cyclopropyl group by resonances at high field, and the carbon–13, as well as the proton NMR, spectra gave no evidence for the presence of olefinic linkages (Table III). These data indicate that compound 4 is a tricyclic system. There were some ambiguities in establishing the stereochemistry at the annulation carbons. MMX[8] calculations showed, however, that the proposed structure was the most stable of four possible stereochemical structures for compound 4. The compound consistent with the NMR and mass spectral data (Scheme I) can be accounted for by the intermediacy of carbocations and participation of the olefinic linkages.

The hydrolysis product identified as compound 5 shows no peak at m/z 470 for the silylated derivative though the PICI mass spectrum with ammonia as reagent gas showed a peak with

Table II. Carbon-13 and Proton NMR Data for Compound 3

Carbon No.	^{13}C Chemical Shifts[a] (ppm)	^{1}H Chemical Shifts[a] (ppm), J. (Hz)
1	38.20	– –
2	[b]	1.76 (dd,1H, J=1.8,11.5), 1.40 (dd,1H, J=2.2,11.2)
3	75.89	4.13 (dd,1H, J=2.2,9.1)
4	136.81	– –
5	128.21	5.05
6	35.53	2.14 (m,1H); 2.63 (m,1H)
7	78.81	3.91 (dd,1H, J=4.1,6.4)
8	156.20	– –
9	44.80	2.51 (dd,1H, J=2.1,10.2), 2.93 (dd,1H, J=5.1,7.4)
10	141.64	4.85 (m,1H)
11	143.70	5.04 (d,1H)
12	22.14	1.11 (s,3H)
13	28.02	0.98 (s,3H)
14	26.50	1.58 (s,3H)
15	114.70	5.22, 5.01 (exomethylene H,2H)

[a] Carbon-13 and proton NMR spectra were obtained in DMSO-d_6.

[b] This ^{13}C spectral line is believed to be under the solvent spectral lines.

Table III. Carbon-13 and Proton NMR Data for Compound 4

Carbon No.	^{13}C Chemical Shifts[a] (ppm)	^{1}H Chemical Shifts[a] (ppm), J. (Hz)
1	33.94	--
2	46.33	1.11 (dd,1H, J=3.2,6.1); 1.39 (dd,1H, J=4.5,8.5)
3	73.16	3.34 (m,1H)
4	74.21	--
5	43.46	0.97 (m,1H)
6	28.96	1.80 (dd,1H, J=3,6.2); 0.50 (m,1H)
7	72.42	3.53 (d,1H, J=6)
8	22.47	--
9	16.41	0.28 (d,2H, J=7.3)
10	22.98	0.49 (m,1H)
11	46.86	0.93 (m,1H)
12	20.46	0.85 (s,3H)
13	24.45	0.92 (s,3H)
14	29.97	1.05 (s,3H)
15	13.74	0.75 (s,3H)

[a] Proton and carbon-13 NMR spectra were obtained in DMSO-d_6.

m/z 488 (9%) which could be the $(M+NH_4)^+$ ion. The low intensity ion with m/z 380 could by the $(M-90)^+$ that would result from loss of trimethylsilanol. There is also a very prominent ion peak with m/z 59 in the EI spectrum of the parent compound which is indicative of the presence of a gem dimethyl tertiary alcohol.

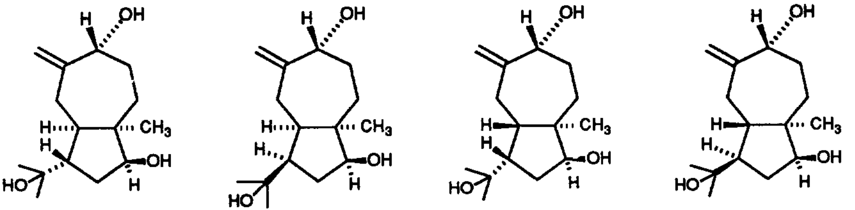
From the ^{1}H–^{13}C heteronuclear correlation spectrum of compound 5 it was observed that there were three carbons (C3, C7, and C11) and two protons (H3, and H7) in the hydroxyl group region. The chemical shift data for the protons and carbon–13s, and the ^{1}H–^{1}H correlation spectrum showing neighboring protons (Table IV), allowed a structure for compound 5 to be proposed. One of the methyl groups of humulene diepoxide A was replaced by an exomethylene group and this is the only double bond in the molecule. As noted above the stereochemistry at C3 and C7 (the two

Table IV. Carbon-13 and Proton NMR Data of Compound 5

Carbon No.	^{13}C Chemical Shift (ppm)	^{1}H Chemical Shift[a] (ppm) J. (Hz)
1	74.06	--
2	35.59	1.50 (m,1H), 1.64 (m,1H)
3	81.40	4.50 (d,1H, J=5.1)
4	[b]	--
5	34.63	1.72 (m,1H); 1.80 (dd,1H, J=3.6,8.1)
6	34.04	1.66 (m,1H); 1.96 (dd,1H, J=4.3,10.4)
7	77.64	3.48 (t,1H, J=8.2)
8	153.90	--
9	39.24	2.17 (d,1H, J=8.0); 2.91 (dd,1H, J=9.9)
10	52.92	1.53 (m,1H)
11	44.20	2.17 (d,1H, J=8.0)
12	27.69	1.15 (s,3H)
13	28.18	1.15 (s,3H)
14	10.87	0.78 (s,3H)
15	111.5	4.70, 4.85 (exomethylene H,2H)

[a] s,dd, m, refer to singlet, doubler of doublet, and multiplet, respectively.

[b] This ^{13}C spectral line is believed to be under the solvent spectral lines.



5a ΔH_f = -19.188 kcal/mol

5b ΔH_f = -79.471 kcal/mol

5c ΔH_f = -84.225 kcal/mol

5d ΔH_f = -88.870 kcal/mol

FIGURE 2. MMX calculated heat of formation for the four possible stereoisomers of compound 5.

carbons bearing the secondary alcohol functionality) should remain the same as in the starting material. The chirality at C1, C4, and C10 cannot be established from the data on hand; however, MMX calculations suggests structure 5d (Figure 2) would be most stable. This compound can arise from the hydrolysis reactions as shown in Scheme I. In the hydrolysis reaction of the 3,4–epoxide, the electrons from the C10–C11 double bond participate and form a bicyclic alcohol with the positive charge at C11. This ion rearranges as shown to form the more stable tertiary carbonium ion which subsequently forms the tertiary alcohol. Bryson and co–workers have discussed this kind of hydrolysis reaction for humulene–3,4–epoxide[9].

The stereochemistry discussed above was established based on mechanistic considerations and NMR data. Conclusive evidence will require X–ray diffraction analysis. Meanwhile it is clear that the hydrolysis of a single stereoisomer in the humulene epoxide series produces a large number of potentially flavor active compounds. Since hops contain at least seven or eight different humulene mono– and diepoxides, the range of products that can be produced from simple hydrolysis reactions is very large indeed.

Aroma/Flavor Profiles of Compounds 1 & 2. Taste panel evaluation of compounds 1 and 2 revealed that the aroma of these compounds is different as expected for diastereomers (Table V). While neither compound is strongly reminiscent of the "noble hop", compound 2 is more likely to contribute a pleasant aroma note than is compound 1. The descriptors for compound 2 include such terms as minty, sweet, floral, fruity, apple, medicinal, etc. Whereas those of compound 1,

TABLE V. Aroma/flavor Evaluation of Compounds 1 and 2

Panelist	Compound 1	Compound 2
1	cardboardy	minty, rancid oil
2	oil	medicine, cleaning fluid
3	rancid oil	apple, sweet, fruity
4	vegetable oil	sweet
5	peanut butter oil	peanut butter, oily, slight floral & medicinal
6	warm water, plastic, dusty	plastic, warm sugar water
7	rancid oil	old floral, musty
8	chemical, rancid oil, cardboard	sweet floral, musty, bubble gum

include frequent reference to rancid, cardboardy, chemical, etc. which are less likely to bring to mind a desirable hoppy character. It is perhaps significant that compound 2 is produced at higher relative (4:1) levels than compound 1. Aroma profiles of the other constituents have not been obtained, but it is certain that the characteristic aroma is produced by blends, synergistically.

ACKNOWLEDGEMENT

We thank Annheuser–Busch Company and the Hop Research Council for financial support. We thank D.A. Griffin for mass spectral data, R.L. Kohnert for NMR spectra, and S. Lindsay and D. McBride for preparation of the manuscript. This is technical paper No. 9165 from the Oregon Agricultural Experiment Station. The Bruker AM 400 NMR spectrometer was purchased with funds from the National Science Foundation (CHE 821690) and from the M.J. Murdock Charitable Trust to OSU. Mass spectral data is available upon request. Registry No. Humulene diepoxide, 40822–04–6.

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