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Tentative Identification of Humulene Diepoxides by Capillary Gas Chromatography/Chemical Ionization Mass Spectrometry

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Five isomers of humulene diepoxide, designated as A-E, were identified by capillary gas chromatography/mass spectrometry (Cap-GC/MS) operated in the pulsed positive ion-negative ion chemical ionization (PPINICI) mode. These isomers had a molecular weight of 236 and a molecular formula of $C_{15}H_{24}O_2$. An herbal/spicy flavor was detected from the mixture of these isomers. These diepoxides were found in selected hop essential oils and in both pilot-scale and commercial beers.

The "noble hop" flavor is generally regarded as one of the most desirable characteristics of beer. In order to achieve this goal, many American breweries import European hops such as Hallertauer mittlefrüher, Northern Brewer, Saazer, and Tettnanger. Earlier studies showed that compounds responsible for hop-derived flavor do not come from the major hydrocarbon fraction, but instead from the minor oxygenated fraction of the hop oil (Howard and Stevens, 1959; Tressl et al., 1978a). Although the argument about the contribution of oxygenated compounds to hoppy aroma in beer has not been settled (Tressl et al., 1978b, 1983; Verzele and Sandra, 1981; Fukuoka and Kowaka, 1983), there is a general agreement that the oxidation products of α -humulene are particularly significant (Tressl et al., 1978a; Peacock et al., 1980; Peacock and Deinzer, 1981). The purpose of this study was to obtain oxidation products of α -humulene through chemical synthesis and to evaluate the contribution from each of these individual oxidation products to the overall beer flavor.

EXPERIMENTAL SECTION

Oxidation of Humulene. *m*-Chloroperbenzoic acid (MCPBA; Aldrich Chemical Co., Milwaukee, WI) was used as the oxidant. In several small portions, 5.8 g (27.1 mmol) of MCPBA was added to a chilled (-15°C) methylene chloride solution containing 5.0 g (24.5 mmol) of α -humulene (Fluka Chemical Co., Hauppauge, NY). The mixture was allowed to stand overnight at 0°C . Unreacted MCPBA and byproduct *m*-chlorobenzoic acid were removed by extracting the methylene chloride solution with 10% sodium hydroxide. The organic solvent was then removed under vacuum on a rotary evaporator. The crude product contained both humulene monoepoxides and diepoxides in a ratio of 4 to 1. When this crude oxidation product was reacted with another molar ratio of MCPBA, a quantitative conversion of humulene to its diepoxides was obtained.

All crude oxidation products of α -humulene were analyzed by capillary gas chromatography/mass spectrometry (Cap-GC/MS).

Hop Essential Oils. Hops were harvested and stored frozen until analysis. Hop essential oils were isolated by the method of Lam et al. (1986a). All samples were analyzed by Cap-GC/MS.

Pilot Beers and Commercial Beers. Four pilot beers were made under identical conditions in a 1-barrel (120-L) pilot-scale brew kettle by a commercial brewery. Normal commercial malt and adjuncts were used. Wort preparation, fermentation, and lagering were performed under standard pilot brewery operations. Three pilot beers were made with one of the three European hop varieties: Hallertauer mittlefrüher, Northern Brewer and Saazer. The raw hops were added 15 min before knockout. The hopping rate was adjusted to produce 30 bitterness units (BU) in the finished product. An unhopped reference brew was adjusted to 30 BU with isomerized α acids.

An American beer of 15 BU and European commercial beer of 40 BU were also chosen for this study.

The extraction and cleanup procedure reported by Lam et al. (1986b) was used to process all beer samples. All extracts were analyzed by Cap-GC/MS.

Cap-GC/MS. All compounds were identified and quantified by a Finnigan Model 4023 quadrupole mass spectrometer. Detailed instrument setup in electron-impact (EI) mode has been reported elsewhere (Lam et al., 1986b). When the mass spectrometer was operated in pulsed positive ion-negative ion chemical ionization (PPINICI) mode, methane was chosen as the reagent gas with a pressure of 0.75 torr at the ion source. A scan range of m/e 45-450 was selected.

RESULTS AND DISCUSSION

Products from chemical epoxidation of α -humulene are shown in Figure 1. Depending on the location and the relative configuration between the two epoxide rings, there could be 12 possible diepoxide isomers. In this study only five isomers were detected among the crude diepoxide mixture. They are temporarily designated isomers A-E

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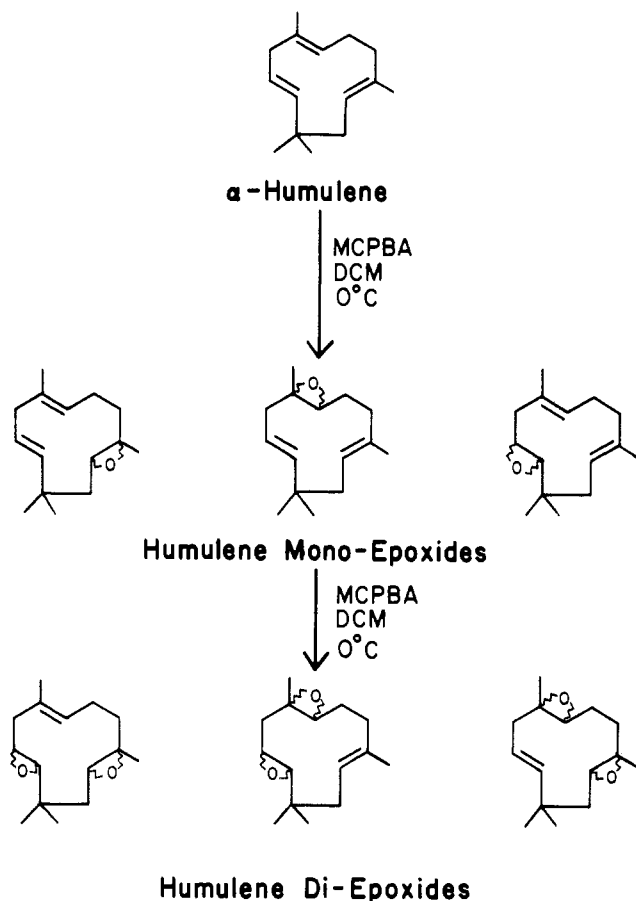


Figure 1. Epoxidation products of α -humulene.

Table I. Chemical Ionization Mass Spectral Data of Humulene Diepoxides

mode	fragment	m/e	humulene diepoxide				
			A	B	C	D	E
PI ^a	(M + H) ⁺	237	6 ^c	4	6	2	4
PI	(M - H) ⁺	235	5	5	nd ^d	1	1
PI	(M + H - H ₂ O) ⁺	219	44	32	nd	24	33
PI	(M + H - 2 H ₂ O) ⁺	201	23	19	nd	18	18
NI ^b	(M - H) ⁻	235	100	100	100	100	100
NI	(M - H - H ₂ O) ⁻	217	28	27	nd	7	1

^aIn positive-ion chemical ionization mode. ^bIn negative-ion chemical ionization mode. ^cRelative abundance (%). ^dNot detected.

until their absolute configuration can be determined.

Electron-impact (EI) mass spectra of these isomers did not produce any useful information, since neither molecular ion nor structural information could be obtained. Low intensities of both protonated and deprotonated molecular ions, (M + H)⁺ at m/e 237 and (M - H)⁺ at m/e 235, were detected in all positive-ion chemical ionization (PICI) mass spectra. In addition, ions related to the loss of one mol-

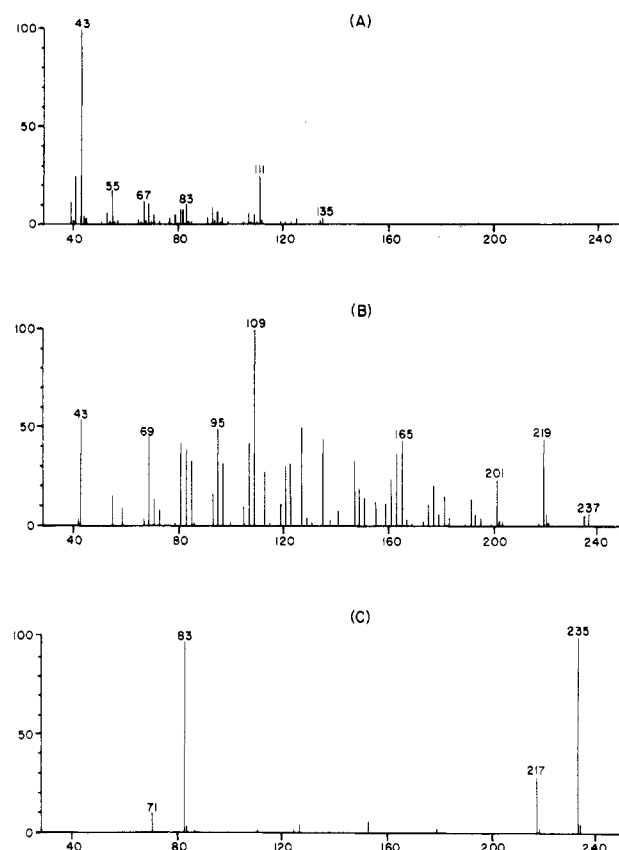


Figure 2. Mass spectra of humulene diepoxide A: (A) electron impact; (B) positive-ion chemical ionization; (C) negative-ion chemical ionization.

ecule of water, (M + H - H₂O)⁺ at m/e 219, and the loss of two molecules of water, (M + H - 2 H₂O)⁺ at m/e 201, from the protonated molecular ion were also observed in all PICI mass spectra. A base peak at m/e 235 was observed for all diepoxide isomers in their negative-ion chemical ionization (NICI) mass spectra, which corresponded to the deprotonated molecular ion, (M - H)⁻. Comparatively, ions corresponding to the loss of a molecule of water from the deprotonated molecular ion, (M - H - H₂O)⁻ at m/e 217, were also observed in all NICI mass spectra. Mass spectral data supported the expected molecular weight of 236 for these diepoxide isomers, and thus the molecular formula of C₁₅H₂₄O₂. Results are summarized in Table I. Mass spectra of humulene diepoxide A in EI, PICI, and NICI modes are compared in Figure 2.

As Ramaswami and Bhattacharyya (1962) had observed, the crude mixture of diepoxides was a thick oil that solidified upon standing. They applied conventional liquid chromatography to isolate the diepoxide fraction and used infrared spectra, optical rotation, and the mixed melting point method to identify the product. Due to the lack of resolution in chromatography and the lack of specificity

Table II. Humulene Diepoxides in Hop Essential Oils

hop variety	crop yr	humulene diepoxides, g/10 g of hops					total
		A	B	C	D	E	
Perle	1982	570.5	129.1	14.7	91.9	31.4	837.5
Perle (imported)	1982	476.7	49.9	45.5	54.5	26.0	652.6
Hallertauer	1982	460.7	60.3	30.6	42.8	21.2	615.5
Hallertauer (imported)	1982	293.8	41.8	25.4	22.6	2.8	386.3
Cascade	1982	402.5	66.0	12.1	33.3	2.6	516.4
Hallertauer	1983	1095.5	159.5	29.3	130.2	43.3	1457.7
Tettnanger	1983	89.9	tr	nd ^a	15.6	17.8	123.3
Cascade	1983	239.9	34.5	tr	12.6	tr	286.9

^a Not detected.

Table III. Humulene Diepoxides in Pilot Beers and Commercial Beers

	humulene diepoxides, $\mu\text{g/L}$ of hops					
	A	B	C	D	E	total
(A) Pilot Beers ^a						
Hallertauer	507.2	88.4	73.5	17.9	nd	687.0
Northern Brewer	259.1	64.3	10.9	98.4	31.0	463.7
Saazer	334.3	53.6	17.5	2.7	nd	408.1
unhopped	nd ^d	nd	nd	nd	nd	nd
(B) Commercial Beers						
I ^b	90.0	17.7	4.6	41.1	11.6	165.0
II ^c	64.5	16.3	nd	nd	nd	80.8

^a Pilot beers brewed with 100% of the specified hop variety. All pilot beers were adjusted to have 30 BU in the finish product. ^b American premium beer of 15 BU. ^c European premium beer of 40 BU. ^d Not detected.

in the method of analysis, they concluded that only one diepoxide isomer was present in the purified product. In our study, a capillary column coupled with a mass spectrometer was used and five isomers of humulene diepoxide were identified. Infrared spectra of diepoxides showed absorption at 1355 and 1380 cm^{-1} corresponding to the gem-dimethyl group and absorption bands at 1240, 1230, 870, 855, 765, and 750 cm^{-1} for two epoxy rings.

Although the oxidation products of α -humulene had long been suspected to impart the noble hop aroma/flavor in beer (Tressl et al., 1978b; Peacock and Deinzer, 1981; Seaton et al., 1982; Moir et al., 1983; Kowaka et al., 1983), the discussion about the contribution of humulene diepoxides to beer flavor was limited (Naya and Kotake, 1972; Tressl et al., 1978b; Peacock and Deinzer, 1981).

Selected hop varieties, pilot beers, and commercial beers were analyzed for humulene diepoxides. Results from the oil analyses of five hop varieties harvested in 2 years are summarized in Table II. All hops analyzed were categorized as aroma hops. Considerable amounts of the five diepoxide isomers, with a total amount ranging from 120 to 1400 $\mu\text{g}/10\text{ g}$ of hops, were detected in all hops.

Results from the analyses of four pilot beers and two commercial beers are shown in Table III. The absence of any diepoxides in the unhopped beer substantiated the fact that these compounds were hop derived. At least four of the five diepoxide isomers were found in all three pilot beers. Their total concentration ranged from 400 to 700 $\mu\text{g}/\text{kg}$. Among the two commercial beers, all five diepoxides were detected in the American beer but only two isomers in the European beer. The total amount of diepoxides in the American beer was twice that of the European beer. Since no information about the type of hops added and their hopping rate was available, it would be difficult at this point to speculate what the cause of difference of the diepoxide level in these two commercial beers might be.

The term "herbal/spicy" is frequently used to describe the noble hop flavor; however, the term is quite ambiguous and often debatable. On the basis of an observation that compounds responsible for the herbal fraction from a beer extract were humulene monoepoxide II, humulenol II, and humulol (Kowaka et al., 1984), a mixture of monoepoxides from our study was used as a reference to evaluate the flavor characteristics of the diepoxides by a trained taste panel. Results revealed that diepoxides showed a very similar but slightly stronger aroma, in comparison to that

of the monoepoxides. In addition, both monoepoxides and diepoxides gave a moderate to strong bitter taste.

From our study, significant amounts of diepoxides have been found in the selected hop essential oils, as well as pilot beers and commercial beers. These diepoxides exhibit similar aroma and taste characteristics to those from monoepoxides, and it is logical to expect these diepoxides might contribute, at least to some extent, to the overall beer flavor. It will be difficult at this moment to fully assess the contribution from each diepoxide isomer, until these isomers have been isolated and purified.

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