Trihydroxyoctadecenoic Acids in Beer: Qualitative and Quantitative Analysis

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Linoleic acid derived 9,10,13- and 9,12,13-trihydroxyoctadecenoic acids present in beer were subjected to stereochemical analysis by a method recently developed (Hamberg, M. *Lipids* **1991**, *26*, 407–415). Although all of the 16 possible isomers were detected, ca. 60% of total 9,10,13- and 9,12,13-trihydroxyoctadecenoates was accounted for by a single isomer, i.e., 9(*S*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid. A method for quantitative determination of 9,10,13- and 9,12,13-trihydroxyoctadecenoic acids was developed. Application of the method to five samples of beer showed that the mean concentration of 9(*S*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid was 5.9 ± 1.1 μg/g and that the total concentration of 9,10,13- and 9,12,13-trihydroxyoctadecenoic acid isomers was 9.9 ± 2.1 μg/g of beer.

INTRODUCTION

The presence of 9,10,13-trihydroxy-11(*E*)-octadecenoic acid in beer was reported 20 years ago (Drost et al., 1971). The finding that trihydroxyoctadecenoic acids were formed from linoleic acid during malting and mashing of barley (Drost et al., 1974) suggested that these processes were responsible for the occurrence of trihydroxy acids in beer. In subsequent work, 9,10,13-trihydroxy-11(*E*)-octadecenoic acid, 9,12,13-trihydroxy-10(*E*)-octadecenoic acid, and 9,10,11-trihydroxy-12(*E*)-octadecenoic acid were identified and quantified in beer (Esterbauer and Schauenstein, 1977a). These workers also found that incubation of linoleic acid with a suspension of barley flour in water led to the formation of trihydroxyoctadecenoic acid isomers in proportions similar to those found in beer (Esterbauer and Schauenstein, 1977b). Trihydroxyoctadecenoic acids have been reported to be bitter-tasting (Baur et al., 1977; Baur and Grosch, 1977; Biermann et al., 1980) and may therefore influence the taste of beers. In addition, trihydroxyoctadecenoic acids have been suggested to play a role in staling of beer (Drost et al., 1974; Esterbauer and Schauenstein, 1977a).

Regio- and stereoisomeric trihydroxyoctadecenoic acids containing the 1,2,5-trihydroxy-3(*E*)-pentene moiety are formed by hydrolysis of allylic epoxy alcohols derived from linoleic acid 9- and 13-hydroperoxides (9*S*-HPOD and 13*S*-HPOD; Figure 1). A method was recently developed for analysis of stereoisomers of 9,10,13- and 9,12,13-trihydroxyoctadecenoates (Hamberg, 1991). This method was used in the present study to determine the percentage composition of stereoisomers of trihydroxyoctadecenoates present in beer. In addition, a method for quantitative analysis of trihydroxyoctadecenoates was developed and used for the determination of these compounds in beer.

MATERIALS AND METHODS

Linoleic acid was purchased from Nu-Chek-Prep, Elysian, MN, and [1-^{14}C]linoleic acid was obtained from Amersham Laboratories, Amersham, U.K. 13*S*-[1-^{14}C]HPOD (specific radioactivity, 1.81 kBq/μmol; enantiomeric composition, 13*S*/13*R*, 99:1) was obtained by incubation of [1-^{14}C]linoleic acid with soybean lipoxygenase (Hamberg and Gotthammar, 1973).

9,10,13- and 9,12,13-Trihydroxyoctadecenoic Acids. Reference samples of 9,10,13- and 9,12,13-trihydroxyoctadecenoic acids of known stereochemistry (compounds **5a–12a**, Figure 2) were prepared as described (Hamberg, 1987, 1991). The preparation of 9(*R*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid

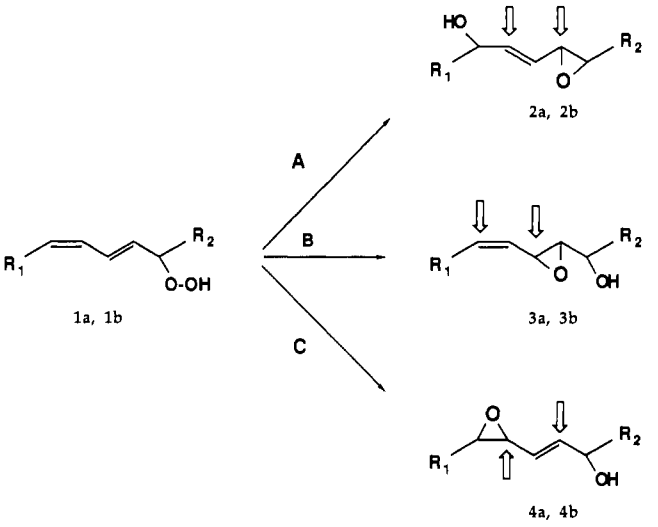

Figure 1. Mechanisms of formation of allylic epoxy alcohols from linoleic acid hydroperoxides. (A) Reaction catalyzed by inter alia ferrous ion, hemoglobin, hematin, and acid; (B) reaction catalyzed by vanadium oxyacetylacetonate and by hydroperoxide isomerase from *Saprolegnia parasitica*; (C) reaction catalyzed by hydroperoxide isomerase from *S. parasitica* and by hydroperoxide-dependent epoxygenase from *Vicia faba*. Arrows indicate positions attacked during solvolysis into trihydroxy acids. **1a**, 9*S*-HPOD; **2a**, **3a**, **4a**, epoxy alcohols formed from 9*S*-HPOD [R_1 = C_6H_{11}, R_2 = $(CH_2)_7COOH$]. **1b**, 13*S*-HPOD; **2b**, **3b**, **4b**, epoxy alcohols formed from 13*S*-HPOD [R_1 = $(CH_2)_7COOH$, R_2 = C_6H_{11}].

(**9a**) described below illustrates the methodology used. 13*S*-[1-^{14}C]HPOD (41.8 mg, 134 μmol) was esterified by treatment with diazomethane in diethyl ether/methanol (9:1 v/v) for 30 s. The methyl ester was treated with hexane (100 mL) saturated with vanadium oxyacetylacetonate at 22 °C for 1 h. This resulted in the formation of a mixture of methyl 11(*R*),12(*R*)-epoxy-13(*S*)-hydroxy-9(*Z*)-octadecenoate and methyl 11(*S*),12(*S*)-epoxy-13(*S*)-hydroxy-9(*Z*)-octadecenoate (ratio, 1:1). The first mentioned epoxy alcohol was isolated by preparative thin-layer chromatography (TLC) [solvent system, ethyl acetate/hexane (25:75 v/v); R_f 0.46] and then rechromatographed by using the same solvent system to remove possible traces of the diastereomeric 11(*S*),12(*S*)-epoxy alcohol. Part of the 11(*R*),12(*R*)-epoxy alcohol was dissolved in dimethoxyethane (2 mL) and treated with water (8 mL) and dilute hydrochloric acid until an apparent pH of 2 was attained. After 5 min at 22 °C, the solution was extracted with diethyl ether. The residue obtained after evaporation of the solvent was subjected to TLC (solvent system, ethyl acetate). Methyl 9(*R*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoate (5 μmol;

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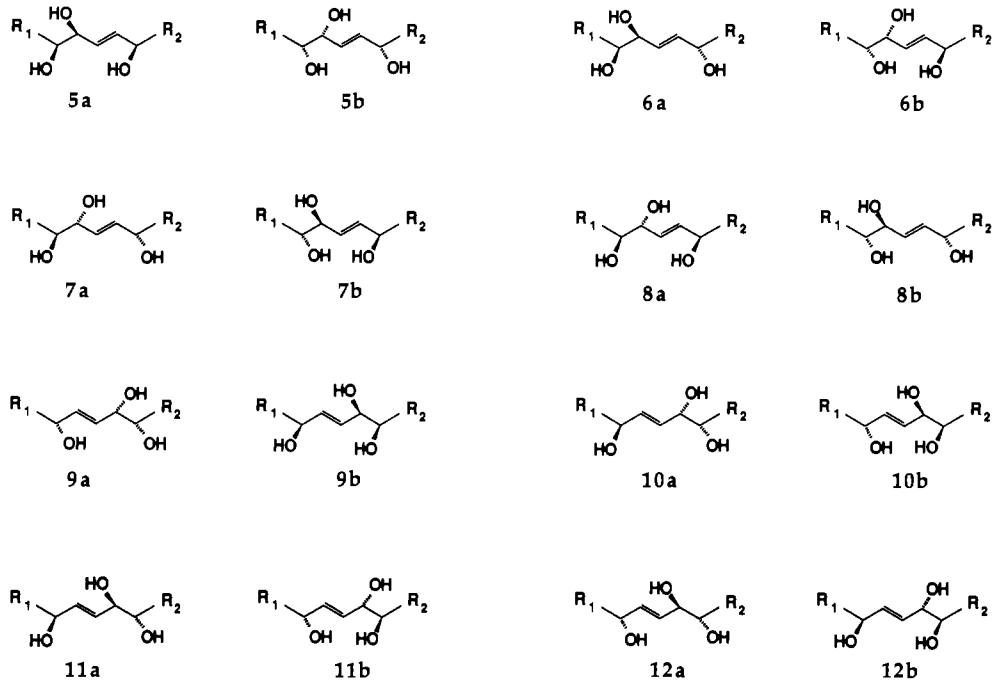

Figure 2. Structures of 9,10,13-trihydroxy-11(*E*)-octadecenoic acids (isomers **5a–8b**) and of 9,12,13-trihydroxy-10(*E*)-octadecenoic acids (isomers **9a–12b**). [$R_1 = (CH_2)_7COOH$, $R_2 = C_5H_{11}$].

R_f 0.39) thus obtained was treated with 40 mL of 0.5 M NaOH in 50% aqueous methanol at 22 °C for 15 h. Material obtained by extraction with ethyl acetate was subjected to silicic acid column chromatography. Elution with ethyl acetate/hexane (6:4 v/v) yielded 9(*R*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid (4.6 μmol). Its identity was confirmed by infrared spectrometry, gas–liquid chromatography–mass spectrometry [GC–MS; trimethylsilyl (Me_3Si) ether derivative of methyl ester], and oxidative ozonolysis performed on the (–)-menthoxycarbonyl (MC) and cyclic carbonate (CC) derivatives as described (Hamberg, 1991).

Extraction of Beer Samples. Beer (50–300 g) was acidified to pH 2 by addition of 2 M hydrochloric acid. Extraction was performed with 2 volumes of chloroform/methanol (2:1 v/v) followed by 1 volume of chloroform. The organic phases were combined, washed with water until neutral reaction, and taken to dryness. The residue was esterified by treatment with diazomethane and subjected to TLC (solvent, ethyl acetate).

Chromatographic and Instrumental Methods. Regular TLC was carried out with precoated plates (Kieselgel 60, 0.25 mm) from E. Merck (Darmstadt, Germany). Separation of erythro and threo isomers of methyl trihydroxyoctadecenoates was achieved with plates coated with sodium arsenite/silica gel G (1:9 w/w) and a solvent system consisting of methanol/chloroform (3:97 v/v). Material was located by spraying with 2′,7′-dichlorofluorescein and viewing under UV light. Silicic acid chromatography was carried out with glass columns packed with silicic acid (Mallinckrodt, Paris, KY; 100 mesh, activated at 120 °C). The columns were eluted under pressure with increasing concentration of ethyl acetate in hexane. Gas–liquid chromatography (GLC) was performed with a Hewlett-Packard Model 5890 gas chromatograph equipped with a methyl silicone capillary column (length, 25 m; film thickness, 0.33 μm). Helium at a flow rate of 25 cm/s was used as the carrier gas. Peak areas were integrated by using a Hewlett-Packard Model 3396A integrator. GC–MS was carried out with a Hewlett-Packard Model 5970B mass selective detector connected to a Hewlett-Packard Model 5890 gas chromatograph. Radioactivity was determined with a Packard Tri-Carb Model 4450 liquid scintillation counter. Infrared spectra were recorded with a Perkin-Elmer Model 257 infrared spectrophotometer.

RESULTS

Regiochemical and Stereochemical Analysis of Trihydroxyoctadecenoic Acids. The percentage composition of isomers **5–12** (Figure 2) in beer was determined by a method recently developed (Hamberg, 1991). Briefly, the lipid extract of 100 g of beer was esterified and subjected to TLC (solvent, ethyl acetate). This resulted in separation of trihydroxyoctadecenoates into two groups, i.e., isomers in which the two allylic, nonvicinal hydroxyl groups were located on either the same or the opposite sides with respect to the plane of the double bond (*cis*-1,4-diols and *trans*-1,4-diols, respectively). The zones of trihydroxyoctadecenoates containing a *cis*-1,4-diol structure (i.e., the methyl esters of compounds **5**, **7**, **9**, and **11**; R_f 0.37–0.39) and of trihydroxyoctadecenoates containing a *trans*-1,4-diol structure (methyl esters of compounds **6**, **8**, **10**, and **12**; R_f 0.47–0.50) were recovered, and methyl ricinoleate (221 μg) was added as an internal standard. The fractions of *cis*- and *trans*-1,4-diols were converted into the Me_3Si derivatives and analyzed by GLC. The percentage amounts of the eight trihydroxy acid isomers **5–12** were calculated from the ratios of the peak areas relative to the peak area of the added methyl ricinoleate internal standard (Hamberg, 1991). As seen in Figure 3A and Table I, the four *cis*-1,4-diol isomers appeared in comparable amounts. On the other hand, the fraction of the four *trans*-1,4-diol isomers was mainly due to the methyl ester of compound **10** (Figure 3B; Table I). The identity of the trihydroxy ester isomers isolated from beer with the methyl esters of the references, i.e., compounds **5–12**, was ascertained by TLC analysis using plain as well as sodium arsenite impregnated silica gel, by GLC analysis and determination of the *C* values of the individual isomers (Hamberg, 1991), and by mass spectrometrical analysis using the authentic isomers as references. Furthermore, the double-bond position as well as the location of the non-glycolic hydroxyl group of the different trihydroxy acid isomers obtained from beer was established by oxidative ozonolysis (Hamberg, 1971) carried out during the steric analysis procedure described below.

As seen in Figure 3B, analysis of the Me_3Si derivatives of the methyl esters of the *trans*-1,4-diol isomers showed the presence of isomers **6**, **8**, **10**, and **12**, as well as an additional compound eluting between isomers **10** and **8**.

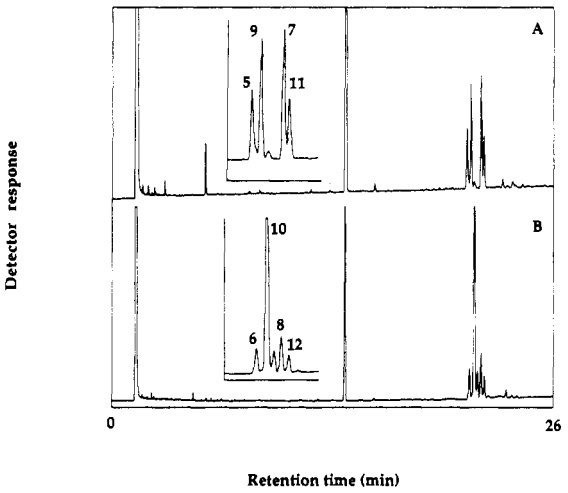

Figure 3. Gas chromatograms of Me_3Si derivatives of the methyl esters of trihydroxy acids **5**, **7**, **9**, and **11** (A) and of trihydroxy acids **6**, **8**, **10**, and **12** (B). Enlarged partial chromatograms of trihydroxy esters are shown by insets. The peak at 13.7 min is due to the Me_3Si derivative of the added methyl ricinoleate standard. Conditions: methyl silicone capillary column (length, 25 m; film thickness, 0.33 μm); carrier gas, helium; flow rate, 25 cm/s; injection port temperature, 260 °C; column temperature, 200 °C raised to 252 °C at 2 °C/min.

The mass spectrum recorded on this material showed ions at inter alia *m/e* 171 (base peak, $Me_3SiO^+{=}CHCH_2CH{=}CHCH_2CH_3$), 259 ($Me_3SiO^+{=}CH(CH_2)_7COOCH_3$), 399 (M − 159; loss of Me_3SiOH plus $^\bullet CH_2CH{=}CHCH_2CH_3$), and 437 (M − 121; loss of Me_3SiOH plus $^\bullet OCH_3$), suggesting a methyl 9,12,13-trihydroxy-10,15-octadecadienoate derived from α-linolenic acid. Selected monitoring of the ions *m/e* 171, 399, and 437 in the gas chromatograms shown in Figure 3 suggested the presence of additional minor isomers of methyl 9,10,13- and 9,12,13-trihydroxyoctadecadienoates derived from α-linolenic acid. Some of these compounds cochromatographed with the linoleic acid derived trihydroxyoctadecenoates; however, the percentage amounts of the compounds relative to the linoleic acid derived trihydroxyoctadecenoates were below 5% and their presence did not significantly affect the determination of trihydroxyoctadecenoate isomers.

The enantiomeric composition of compounds **5**–**12** was determined by steric analysis of the MC derivatives of methyl 2-hydroxyheptanoate and dimethyl 2-hydroxysebacate obtained following oxidative ozonolysis (Hamberg, 1971, 1991). Briefly, the lipid extract of 300 g of beer was esterified and subjected to TLC (solvent, ethyl acetate). The fractions of *cis*- and *trans*-1,4-diols were subjected to sodium arsenite TLC [solvent, methanol/chloroform (3:97 v/v)]. In this way four fractions were obtained, i.e., *threo*/*cis*-1,4-diols (methyl esters of **5** and **9**), *threo*/*trans*-1,4-diols (methyl esters of **6** and **10**), *erythro*/*cis*-1,4-diols (methyl esters of **7** and **11**), and *erythro*/*trans*-1,4-diols (methyl esters of **8** and **12**). Treatment of these materials with (−)-menthoxycarbonyl chloride resulted in the formation of tris-MC derivatives which were purified by TLC and subjected to oxidative ozonolysis. The configurations of C-13 of **5**–**8** and of C-9 of **9**–**12** followed from gas chromatographic analysis of the MC derivatives of methyl 2-hydroxyheptanoate and dimethyl 2-hydroxysebacate, respectively. Table I gives the enantiomeric compositions of compounds **5**–**12** as well as the percentages of the enantiomers of compounds **5**–**12** found in analyses of five samples of beer.

Quantitative Determination of Trihydroxyoctadecenoic Acids in Beer. 9(*R*),12(*S*),13(*S*)-Trihydroxy-10-(*E*)-octadecenoic acid (compound **9a**; 61.1 μg) in ethanol (1 mL) was added to a weighed sample of beer (about 50 g). The fraction of methyl trihydroxyoctadecenoates containing the *cis*-1,4-diol structure was isolated by TLC and analyzed by GLC as the Me_3Si derivatives. The percentage amount of **9** relative to the total amount of *cis*-1,4-diols (**5** + **7** + **9** + **11**) (=*B*) was determined. The percentage amount of compound **9** relative to the total amount of *cis*-1,4-diols (**5** + **7** + **9** + **11**) (=*A*) in the same beer analyzed without addition of **9a** was obtained during the regio- and stereochemical analysis described above (cf. Figure 3A). This analysis also provided the percentage amount of *cis*-1,4-diols (**5** + **7** + **9** + **11**) relative to the total amount of trihydroxyoctadecenoates (**5** + **6** + **7** + **8** + **9** + **10** + **11** + **12**) (=*C*). From percentages *A*, *B*, and *C*, and knowledge about the amount of **9a** added (61.1 μg) and the weight of the beer sample to which **9a** was added (=*W*, grams), it was possible to calculate the concentration of total trihydroxyoctadecenoates (=*T*, micrograms per gram of beer) by using the expression

$$T = 61.1(100 - B)/(B - A) \times 100/C \times 1/W$$

Table II shows the results of quantitative determination of trihydroxyoctadecenoates in five samples of beer. The mean concentration of 9,10,13- plus 9,12,13-trihydroxyoctadecenoates was 9.9 ± 2.1 μg/g and that of the major trihydroxy acid isomer, 9(*S*),12(*S*),13(*S*)-trihydroxy-10-(*E*)-octadecenoic acid, was 5.9 ± 1.1 μg/g of beer.

DISCUSSION

Trihydroxyoctadecenoic acids containing the 1,2,5-trihydroxy-3(*E*)-pentene structure are formed from linoleic acid via linoleic acid 9- and 13-hydroperoxides and allylic epoxy alcohols. The initial reaction involves lipoxygenase-catalyzed oxygenation of linoleic acid to produce 9(*S*)-hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid and/or 13-(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid. As shown in Figure 1, conversion of hydroperoxides into epoxy alcohols can occur by several mechanisms. Reaction A leads to the formation of epoxy alcohols containing the *trans*-4,5-epoxy-1-hydroxy-2(*E*)-pentene structure and is catalyzed by several agents including ferrous ion (Gardner et al., 1974), hemoglobin (Hamberg, 1975), and hematin (Dix and Marnett, 1985) and by acid in a protic solvent (Gardner et al., 1984). Reaction B involves conversion of hydroperoxides into epoxy alcohols containing the *trans*-2,3-epoxy-1-hydroxy-4(*Z*)-pentene structure and takes place by intermolecular epoxidation in the presence of vanadium oxyacetylacetonate (Hamberg, 1987), as well as by intramolecular epoxidation catalyzed by a fungal hydroperoxide isomerase (Hamberg et al., 1986; Hamberg, 1989). Epoxy alcohols formed according to route C contain the *cis*-4,5-epoxy-1-hydroxy-2(*E*)-pentene structure and are formed from hydroperoxides in the presence of either hydroperoxide isomerase (Hamberg et al., 1986) or a hydroperoxide-dependent epoxygenase recently described (Hamberg, 1990). In addition, certain cereal flours have been reported to contain an isomerase activity that catalyzes conversion of hydroperoxides into epoxy alcohols containing the *cis*-4,5-epoxy-1-hydroxy-2(*E*)-pentene structure (Graveland, 1970; Heimann and Dresen, 1973). Allylic epoxy alcohols are rapidly hydrolyzed into isomeric trihydroxy acids, especially in an acidic medium (Gardner et al., 1984; Hamberg et al., 1986). Hydrolysis of epoxyhydroxyoctadecenoic acids possessing either a *trans*-4,5-epoxy-1-hydroxy-2(*E*)-pentene structure (**2a** and **2b** in Figure 1) or a *cis*-4,5-epoxy-1-hydroxy-2(*E*)-pentene structure (**4a** and **4b**) occurs by solvent attack at either the allylic epoxide carbon or the olefinic carbon β to the

Table I. Regio- and Stereochemical Analysis of Trihydroxyoctadecenoic Acids Isolated from Five Samples of Beer[a]

compd	percentage amount	ratio 9R/9S	ratio 13R/13S	percentage composition of stereoisomers
5	3.4 ± 0.5		67:33 (±6)	**5a**, 2.3 ± 0.5; **5b**, 1.1 ± 0.1
6	6.1 ± 0.5		14:86 (±2)	**6a**, 5.2 ± 0.5; **6b**, 0.9 ± 0.1
7	6.7 ± 1.3		13:87 (±1)	**7a**, 5.8 ± 1.2; **7b**, 0.9 ± 0.1
8	9.5 ± 2.3		77:23 (±5)	**8a**, 7.4 ± 2.2; **8b**, 2.1 ± 0.2
9	5.7 ± 0.1	64:36 (±3)		**9a**, 3.6 ± 0.2; **9b**, 2.1 ± 0.2
10	60.6 ± 4.7	2:98 (±0)		**10a**, 59.5 ± 4.6; **10b**, 1.1 ± 0.3
11	3.4 ± 0.3	29:71 (±2)		**11a**, 2.4 ± 0.2; **11b**, 1.0 ± 0.1
12	4.6 ± 0.2	36:64 (±10)		**12a**, 1.7 ± 0.4; **12b**, 2.9 ± 0.5

[a] Numbers are means ± SD.

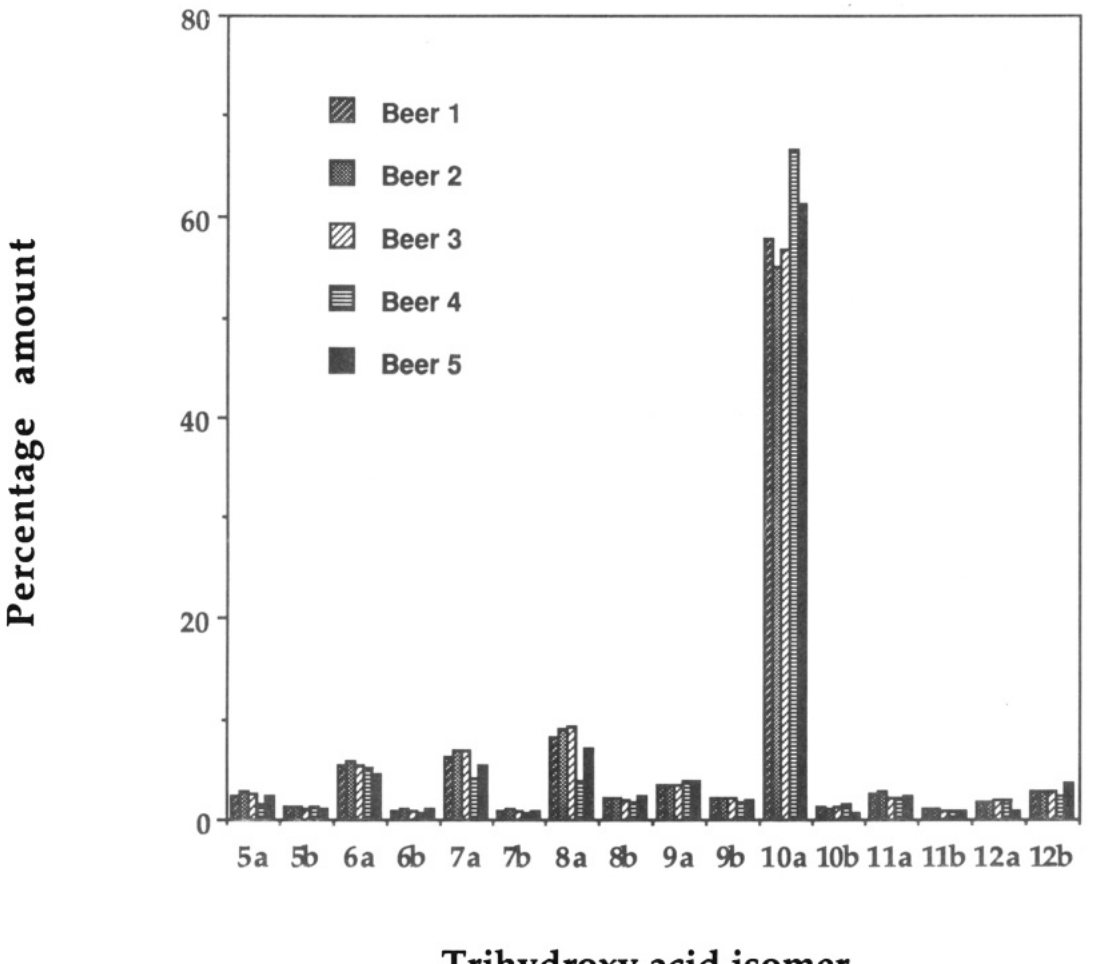

Figure 4. Percentage amounts of trihydroxyoctadecenoic acid isomers **5a–12b** in five samples of beer. Numbering of the beer samples corresponds to the numbering in Table II.

Table II. Quantitative Determination of 9,10,13- and 9,12,13-Trihydroxyoctadecenoic Acids in Five Samples of Beer

beer sample	*A*,[a] %	*B*,[b] %	*C*,[c] %	*W*,[d] g	*T*,[e] μg/g
1 (Swedish)	30.7	54.0	19.9	50.5	12.0
2 (Swedish)	26.5	53.7	21.3	55.4	8.8
3 (Danish)	27.7	50.7	19.9	53.4	12.3
4 (Czech)	35.3	65.0	16.0	53.1	8.5
5 (Dutch)	30.7	60.2	18.8	55.3	7.9

[a] *A*, percentage amount of 9 relative to amount of 5 + 7 + 9 + 11 in original beer sample. [b] *B*, percentage amount of 9 relative to amount of 5 + 7 + 9 + 11 in beer sample after addition of 61.1 μg of 9a. [c] *C*, percentage amount of 5 + 7 + 9 + 11 relative to amount of 5 + 6 + 7 + 8 + 9 + 10 + 11 + 12 in original beer sample. [d] *W*, weight of beer samples analyzed. [e] *T*, sum of concentrations of 5 + 6 + 7 + 8 + 9 + 10 + 11 + 12.

epoxide and results in the formation of several isomers of 9,10,13- and 9,12,13-trihydroxyoctadecenoic acids (Gardner et al., 1984; Claeys et al., 1985; Hamberg, 1989). Such trihydroxy acids are the major products formed also from epoxy alcohols containing the *trans*-2,3-epoxy-1-hydroxy-4(*Z*)-pentene structure (**3a** and **3b**); however, in this case 9,10,11- and 11,12,13-trihydroxyoctadecenoic acids are produced as well (Hamberg, 1987).

Regio- and stereochemical analysis of trihydroxyoctadecenoate carried out in the present study revealed that a single stereoisomer, i.e., 9(*S*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid (**10a** in Figure 2) accounted for ca. 60% (59.5 ± 4.6%) of the 16 9,10,13- and 9,12,13-trihydroxyoctadecenoic acid isomers in beer. Although the mode of formation of this compound is unknown, a probable pathway consists of the sequence linoleic acid → 9(*S*)-hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid (**1a**) → 12(*R*),13(*S*)-epoxy-9(*S*)-hydroxy-10(*E*)-octadecenoic acid (one of the stereoisomers of compound **4a**) → 9(*S*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid (**10a**). This sequence is supported by the recent finding that epoxy alcohols containing the *cis*-4,5-epoxy-1-hydroxy-2(*E*)-pen-

tene moiety are hydrolyzed predominantly by solvent attack at the allylic epoxide carbon with inversion of the configuration of that carbon (Hamberg, 1991) and is in agreement with the fact that the lipoxygenase of ungerminated barley seed catalyzes oxygenation at C-9 of linoleic acid to produce 9(*S*)-hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid (Gardner, 1988; van Aarle et al., 1991).

The method for quantitative determination of the eight separable isomers of 9,10,13- and 9,12,13-trihydroxyoctadecenoic acids (compounds **5–12**) developed in the present study was based on gas chromatographic determination of the percentage amount of 9(*R*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid relative to total trihydroxyoctadecenoic acid isomers. The determination was carried out twice, first with the original beer sample and second with a weighed beer sample to which had been added a known amount of 9(*R*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid. An obvious drawback with this method was the need to carry out the analytical sequence twice; however, this was compensated by the fact that no internal standard unrelated to the trihydroxy acids had to be added. Thus, possible complications caused by nonidentical recovery of compounds during extraction and by different flame ionization detector responses of compounds during GLC were avoided. The total concentration of 9,10,13- and 9,12,13-trihydroxyoctadecenoic acids in five samples of beer found by using this method was 9.9 ± 2.1 μg/g of beer. This result is in agreement with data previously reported, i.e., 5.7–11.4 μg/mL in five different beers (Esterbauer and Schauenstein, 1977a).

A mixture of 9,10,13- and 9,12,13-trihydroxyoctadecenoic acids produced by incubation of linoleic acid with a preparation from soybean has been reported to elicit bitter taste (Baur et al., 1977). The taste threshold was 0.6–0.9 μmol/mL. It should be pointed out that this value was obtained by using a mixture of regio- and stereoisomeric trihydroxyoctadecenoates and that the individual trihydroxy acid isomers may have quite different taste thresholds. Thus, although the total concentration of 9,10,13- and 9,12,13-trihydroxyoctadecenoates in beer was considerably lower (ca. 0.03 μmol/mL) than the above-mentioned taste threshold concentration, it cannot be ruled out that isomer(s) of trihydroxyoctadecenoic acids present in beer may contribute to the bitter taste of beers. In particular, it seems worthwhile to examine the bitter-tasting effect and taste threshold of the major trihydroxy acid isomer, i.e., 9(*S*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid.

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