

Figure 1. Desiccator modified for gas analysis.

baric, modified atmosphere storage and testing of foods.

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## Identification and Quantitation of 6-Hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline in Alcoholic Beverages

A gas chromatographic-mass spectrometric method was used to identify and quantitate 6-hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline (6OHTHBC) in beer. The concentration of 6OHTHBC varied between 33 and 235 nmol/L and was not a function of the alcoholic content of the beer.

6-Hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline (6OHTHBC) is a tricyclic indole derivative which is formed by a Pictet-Spengler reaction involving serotonin (5-hydroxytryptamine, 5-HT) and formaldehyde. The formation of such tetrahydro- $\beta$ -carbolines occurs readily under physiological conditions (Whaley and Govindachari, 1951) and produces substances which can function as neurotransmitters and/or neuromodulators (Buckholtz, 1980). Furthermore, acute and chronic administration of tetrahydro- $\beta$ -carbolines to rats has been reported to significantly alter ethanol consumption (Geller et al., 1973; Myers and Oblinger, 1977). Recently 1-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline and 6-hydroxy-1-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline have been identified and quantitated in various foods and alcoholic beverages (Beck and Holmstedt, 1981; Beck et al., 1983). In light of their pharmacological activity and potential role in alcoholism, we undertook the analysis of 6OHTHBC in alcoholic beverages, which is the subject of this communication.

#### EXPERIMENTAL SECTION

**Materials.** 6-Hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline (6OHTHBC) 5-hydroxytryptamine and glyoxylic acid by the method of Vejdek et al. (1961). The deuterated

internal standard was prepared by the exchange labeling procedure of Elliott (1975). The procedure involved dissolving 100 mg of 6OHTHBC in 10 mL of  $^2\text{H}_2\text{O}$  and 5 mL of  $^2\text{HCl}$  (38% in  $^2\text{H}_2\text{O}$ ) and refluxing for 1 h. The reaction mixture was lyophilized to dryness, and labile deuterium atoms were exchanged by repeated dissolving in  $\text{H}_2\text{O}$  and lyophilization. This procedure gave a mixture of di-deuterated-pentadeuterated molecules; however, the major species and the one monitored in this study was 6OHTHBC- $^2\text{H}_4$ . The positions of deuterium atom labeling have not been established. 5-Hydroxytryptamine creatinine sulfate and 5-methoxytryptamine were obtained from Sigma Chemical Co. (St. Louis, MO), dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) was from E. Merck (Darmstadt, West Germany), and pentafluoropropionic anhydride (PFPA) was from Massanalyt AB (Bromma, Sweden). All other chemicals were of analytical purity.

**Sample Preparation.** A 1.0-mL aliquot of each alcoholic beverage was added to acid-washed (dichromate-sulfuric acid), silanized, glass tubes containing a solution (150  $\mu\text{L}$ ) of 6OHTHBC- $^2\text{H}_4$  (110 pmol), semicarbazide (4.5 mmol), ascorbic acid (0.5 nmol) and EDTA (0.05 nmol). Semicarbazide was added to control for the artifactual formation of 6OHTHBC during the sample workup. Its

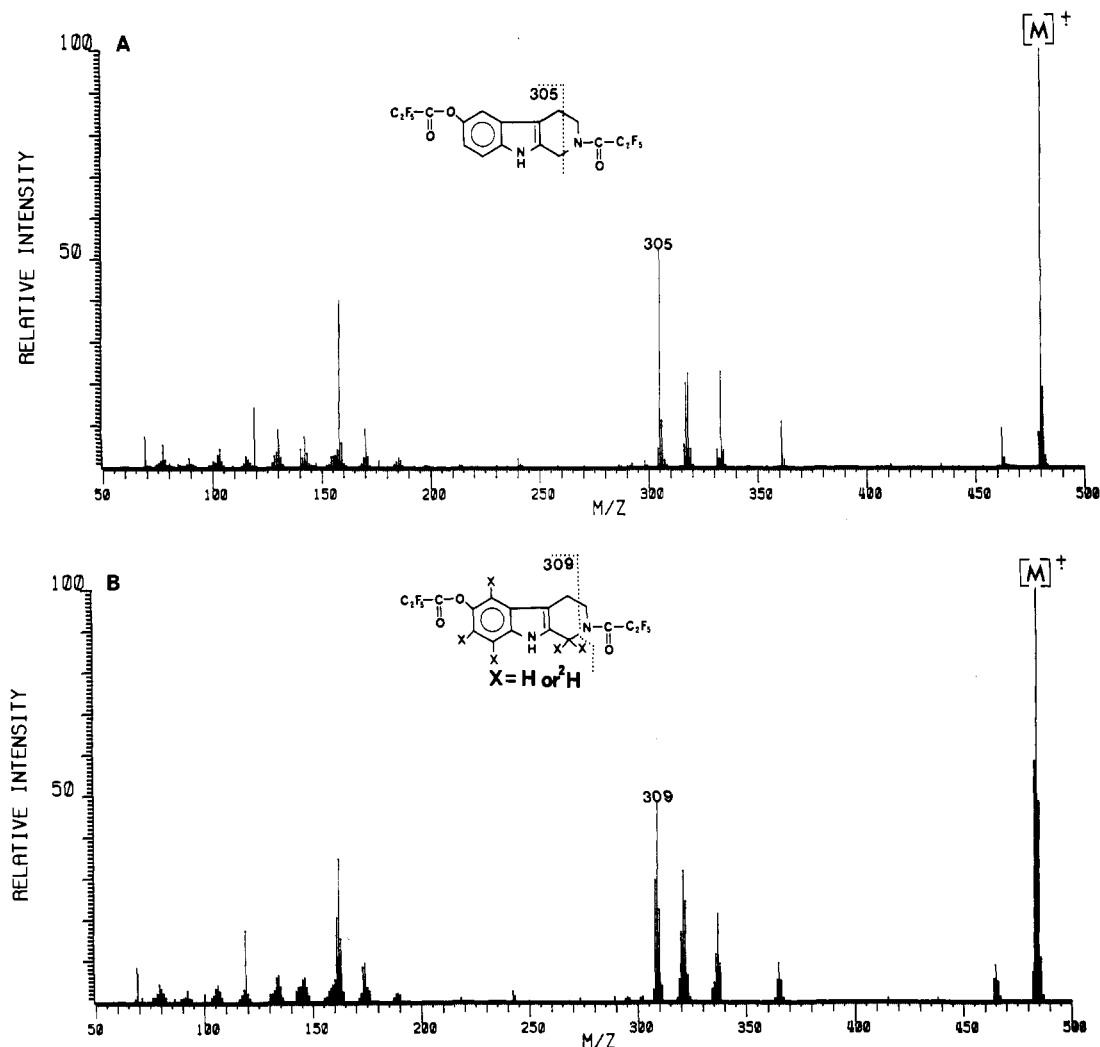


Figure 1. Electron impact mass spectra of the pentafluoropropionyl derivatives of (A) 6OHTHBC and (B) 6OHTHBC-<sup>2</sup>H<sub>4</sub>.

effectiveness was confirmed by the absence of 6OHTHBC-<sup>2</sup>H<sub>4</sub> formation when 5-HT-<sup>2</sup>H<sub>4</sub> was added to the sample prior to workup (Bosin et al., 1982). The samples were treated with 0.4 g of NaCl and 0.1 mL of 5.8 M K<sub>2</sub>CO<sub>3</sub> prior to extraction with 6.0 mL of "formaldehyde-free" CH<sub>2</sub>Cl<sub>2</sub>. The extraction efficiency was greater than 80%. Formaldehyde-free CH<sub>2</sub>Cl<sub>2</sub> was prepared by refluxing 100 mL of CH<sub>2</sub>Cl<sub>2</sub> with 30 mg of 5-methoxytryptamine for 4 h, stirring at room temperature overnight, and distilling immediately prior to use (Bosin et al., 1982). The organic layers were transferred to new tubes and evaporated to dryness under nitrogen. The residues were derivatized with 50 μL of PFP for 20 min at 60 °C. After being cooled, the excess reagent was evaporated under nitrogen and the residues were dissolved in 25 μL of ethyl acetate.

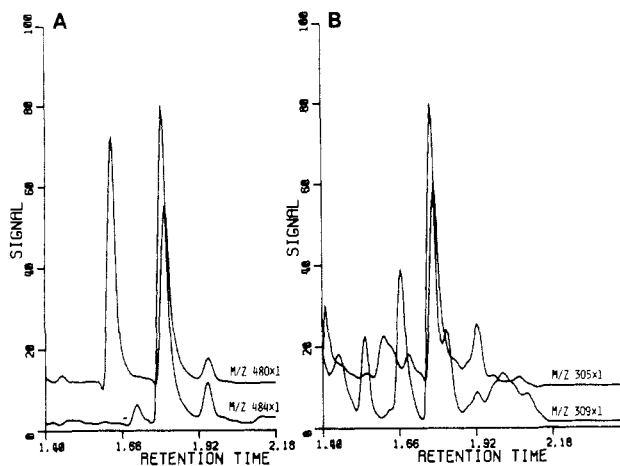
**Gas Chromatography–Mass Spectrometry.** A computer-controlled LKB 2091 gas chromatograph–mass spectrometer was used for multiple ion detection and recording of mass spectra. The gas chromatographic separations were achieved with a 20-m SE52 WCOT glass capillary column (i.d. 0.30 mm) by using helium as a carrier and makeup gas. Splitless injections were carried out by using a moving needle device (van den Berg and Cox, 1972). The gas chromatographic conditions were as follows: injector heater 280 °C, column temperature 250 °C, column flow rate 2 mL/min, and makeup gas flow rate 12 mL/min. Aliquots (2 μL) of the samples were injected and an initial delay of 1.4 min in opening the separator valve

was used to avoid contamination of the ion source. Under these conditions, the retention time of 6OHTHBC-(PFP)<sub>2</sub> and the deuterated analogue was approximately 1.8 min. The mass spectrometric conditions were as follows: separator temperature 270 °C, ion source temperature 240 °C, electron energy 70 eV, and trap current 50 μA.

**Quantitation.** Calibration curves were constructed by plotting the peak height ratios (*m/z* 480/484) of standard samples against the 6OHTHBC concentration. The standard samples were prepared by adding varying amounts of 6OHTHBC (0–239 pmol) to glass tubes containing 1.0 mL of H<sub>2</sub>O and 6OHTHBC-<sup>2</sup>H<sub>4</sub> (110 pmol). All samples were carried through the extraction procedure (vide supra). The 6OHTHBC levels were determined from the peak height ratios of each sample by reference to the calibration curve. The limit of sensitivity of the method was less than 5 pmol of 6OHTHBC/sample. The experimental error determined for a series of 6OHTHBC assays (15.8 pmol/sample, *N* = 8) was 4.7%.

## RESULTS AND DISCUSSION

The electron impact mass spectra of the PFP derivatives of authentic 6OHTHBC (Figure 1A) and 6OHTHBC-<sup>2</sup>H<sub>4</sub> (Figure 1B) indicated the presence of two PFP groups per molecule; no evidence for a (PFP)<sub>3</sub> derivative was obtained. The compounds produced abundant molecular ions at *m/z* 480 and 484, respectively, which represented the base peaks. The peaks at *m/z* 305 and 309 result from a retro Diels–Alder fragmentation pathway. The multiple ion



**Figure 2.** Chromatograms obtained from the analysis of Norrland guld beer: (A)  $m/z$  480/484; (B)  $m/z$  305/309. Mass members and relative gain amplification factors are indicated on the figure.

**Table I.** Concentration of 6-Hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline in Beer

beer <sup>a</sup>	alcohol content, % (w/w)	concn of 6OHTHBC, nmol/L
Coop dark I	1.8	38
Coop light I	1.8	57
Kavaljer I	1.8	63
Tre Towns I	1.8	115
Pripps Blå I	1.8	125
Pilsner II	2.8	16
Tuborg II	2.8	33
Porter II	2.8	37
Warby Special II	2.8	69
Pripps Blå II	2.8	103
Norrland Guld II	2.8	112
Tre Towns II	2.8	114
Bass III	4.5	37
Guinnes III	4.5	117
Tre Towns III	4.5	146
Falcon III	4.5	235

<sup>a</sup> All from Sweden except Tuborg (Denmark), Bass (England), and Guinnes (Ireland).

detection was performed by monitoring ion intensities at these four mass numbers.

The identification of 6OHTHBC in these samples was based on the presence of peaks at both  $m/z$  480 and  $m/z$  305 with the same retention time and ion intensity ratios as authentic and tetradeuterated 6OHTHBC (Figure 2). When 6OHTHBC-<sup>2</sup>H<sub>4</sub> was analyzed as described under Experimental Section, the internal standard and reagent yielded a 6OHTHBC blank value of 1.5 pmol/sample. A typical blank chromatogram has been previously published (Bosin et al., 1982).

Various alcoholic beverages were analyzed for 6OHTHBC by monitoring the ion intensity ratio  $m/z$

480/484. Only in beer was 6OHTHBC present in detectable and quantifiable amounts (Table I). In contrast to the levels of 6-hydroxy-1-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline (6OMTHBC) which has been reported to vary directly with the alcoholic content of beer (Beck et al., 1983), the levels of 6OHTHBC showed no such relationship. The correlation of 6OMTHBC levels with alcoholic content and their generally higher levels in beer than 6OHTHBC may reflect a greater concentration of acetaldehyde vs. formaldehyde and thus a greater opportunity for formation via a Pictet-Spengler reaction. Although the origin of 6OHTHBC in beer is unknown, the presence of similar 6OHTHBC levels in the various classes of Tre Towns and Pripps Blå beers suggests that the source may be the brewing ingredients. Since tetrahydro- $\beta$ -carbolines possess significant pharmacological activity, their presence in beer may have behavioral and toxicological implications for the consumer.

**Registry No.** 6-Hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline, 23778-34-9.

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