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Ethyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate: a novel β-carboline found in alcoholic beverages

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Abstract

A novel tetrahydro-β-carboline, chemically identified as 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester (ethyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate) (MTCA-EE), has been found in alcoholic beverages ranging from an undetectable amount to 534 μg/l. RP-HPLC with fluorescence detection and GC–MS were used for characterization. MTCA-EE occurred as two diastereoisomers 1*S*,3*S* and 1*R*,3*S* with an average ratio (*SS*/*RS*) of 2.21. The concentration of MTCA-EE (1*S*,3*S* plus 1*R*,3*S*) was 109.6–534 μg/l in sherry wines, 75.2–140.7 μg/l in sparkling wines, 3.4–161.65 μg/l in red and white wines, 32.7–47.2 μg/l in port wines, and 8.7–37.2 μg/l in sake. MTCA-EE seemed to occur in some high alcohol beers at less than 10 μg/l. Most distilled alcoholic beverages did not contain MTCA-EE although, exceptionally, one brandy reached 28.6 μg/l. 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) and L-tryptophan ethyl ester (L-TRP-EE) were two precursors leading to MTCA-EE. Alcoholic drinks containing MTCA-EE and MTCA could be an exogenous source of bioactive β-carbolines found in vivo. MTCA-EE is a structural analogue (reduced pyrido ring) of β-carboline-3-carboxylic acid ethyl ester (β-CCE), a potent benzodiazepine receptor inverse agonist.

1. Introduction

1,2,3,4-Tetrahydro-β-carbolines (THβCs) (2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole) are naturally occurring indole derivatives formed from indole ethylamines and aldehydes and/or alpha-ketoacids through Pictet–Spengler condensation. Similarly, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (THβC-3-COOHs) arise from L-tryptophan and aldehydes (Scheme 1). This reaction occurs under mild conditions and the rate is temperature- and pH-dependent (Herraiz & Ough, 1993).

Tetrahydro-β-carbolines and β-carbolines have attracted the attention of neurochemists who have pointed out their occurrence under physiological conditions in biological tissues and fluids (Adachi, Mizoi, Naito, Ogawa, Uetani & Ninomiya, 1991a; Airaksinen & Kari, 1981a; Brossi, 1993; Buckholz, 1980; Callaway, Gynther, Poso, Vepsäläinen & Airaksinen, 1994; Melchior & Collins, 1982; Myers, 1989; Rommelspacher, May & Susilo, 1991). This has encouraged speculation on their putative role in the central nervous system where they could function as neuromodulators via effects on the monoamine oxidase, monoamine uptake and benzodiazepine receptor binding (Airaksinen & Kari, 1981a,b; Buckholtz, 1980; Melchior & Collins, 1982; Myers, 1989; Rommelspacher et al., 1991). Simultaneously, they have been increasingly studied in relation to alcoholism (Adachi, et al., 1993; Beck, Bosin, Holmstedt & Lundman, 1982; Cohen & Collins, 1970; Myers, 1989; Myers & Melchior, 1977; Rommelspacher & Schmidt, 1985; Tuomisto, Airaksinen, Peura & Eriksson, 1982). Some β-carbolines are comutagens or precursors or mutagens (Higashimoto, Yamamoto, Kinouchi, Matsumoto & Ohnishi, 1996; de Meester, 1995; Wakabayashi et al., 1983), can cause neuronal cell death in vitro (Brenneman et al., 1993), and can be bioactivated, giving rise to endogenous neurotoxins (Albores, Neafsey, Drucker, Fields & Collins, 1990; Collins & Neafsey, 1985).

We have reported that two tetrahydro-β-carbolines, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA), are extensively present in commercial foods and drinks, and suggested that tetrahydro-β-carbolines in biological tissues, could arise from dietary sources (Herraiz, 1996, 1997, 1998; Herraiz, Huang & Ough, 1993; Herraiz & Ough, 1993; Herraiz & Sanchez, 1997). Also, tetrahydro-β-carbolines

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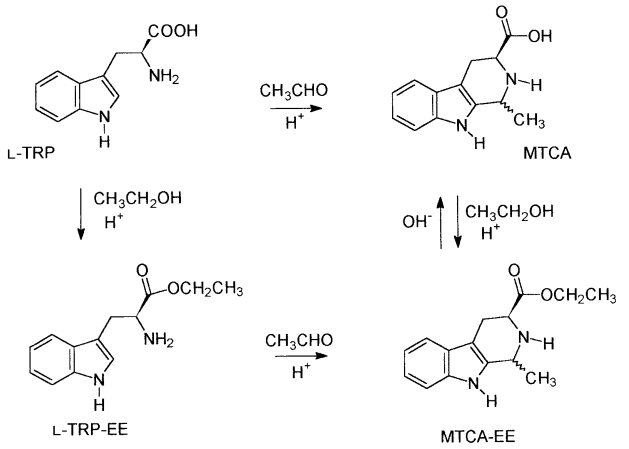

Scheme 1. Proposed routes for the formation of MTCA-EE from L-TRP, MTCA and L-TRP-EE. 1,3-Disubstituted-tetrahydro-β-carbolines, such as MTCA, and MTCA-EE appear as a mixture of 1*S*,3*S* and 1*R*,3*S* diastereoisomers.

might be formed endogenously, for example, after alcohol ingestion, through its metabolite acetaldehyde or in pathological states (Adachi et al., 1991b; Adachi et al., 1993). In this regard, β-carbolines might play a role in the aetiology or addiction of alcoholism (Adachi et al., 1993; Adell & Myers, 1994; Beck et al., 1982; Myers, 1989; Myers & Melchior, 1977; Rommelspacher et al., 1991; Rommelspacher & Schmidt, 1985). Obviously, any possible presence of these compounds in alcoholic beverages should be taken into consideration. MTCA and THCA were found in wines and beers reaching up to several mg per litre (Bosin, Krogh & Mais, 1986; Gutsche & Herderich, 1997; Herraiz, 1996; Herraiz et al., 1993; Herraiz & Ough, 1993, 1994; Sen, Seaman, Lau, Weber & Lewis, 1995); however, the possible presence of their ethyl esters derivatives has not been investigated so far. Esters of THβC-3-COOHs are tetrahydro structural analogs (reduced pyrido ring) of esters of β-carboline-3-carboxylic acids that bind with very high affinity to the benzodiazepine receptor and exhibit proconvulsant and anxiogenic properties (Braestrup, Nielsen & Olsen, 1980; Cain et al., 1982; Ninan et al., 1982; Tenen & Hirsch, 1980). This paper reports, for the first time, the identification and occurrence of ethyl esters of THβC-3-COOHs in alcoholic beverages and briefly discusses their origin, and possible biological implications.

2. Materials and methods

2.1. Reference and synthesized compounds

Tryptamine, L-tryptophan, L-tryptophan methyl ester hydrochloride, L-tryptophan ethyl ester hydrochloride (TRP-EE), were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) was purchased from Sigma, and also synthesized from L-tryptophan and acetaldehyde giving rise to two diastereoisomers (1*S*,3*S*, major compound and 1*R*,3*S*, minor compound) (Adachi et al., 1991a; Brossi, Focella & Teitel, 1973; Herraiz & Ough, 1993, 1994). Mass spectrum (VG 12-250, MassLab, Altrincham, UK, 70 EV), *m*/*z* (% rel. abundance): 230 (M^+, 55), 215 (81), 157 (100), 169 (90). 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid methyl ester (MTCA-ME) was prepared from L-tryptophan methyl ester hydrochloride and acetaldehyde as previously (Herraiz, 1997). MTCA-ME appears as a mixture of 1*S*,3*S* and 1*R*,3*S* diastereoisomers (ca 9:1 ratio) and 99% of purity by HPLC. MS (70 EV), *m*/*z* (%): 244 (M^+ 61), 229 (61), 157 (100), 169 (80). 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester (MTCA-EE) was prepared from L-tryptophan ethyl ester hydrochloride (TRP-EE) and acetaldehyde stirred at room temperature until formation of a precipitate, that was redissolved in ethanol, treated with drops of conc HCl, and evaporated to give MTCA-EE (Herraiz, 1997). MTCA-EE appears in a diastereomeric ratio (1*S*,3*S*/1*R*,3*S*) of 36 (ca 99% purity by HPLC). MS (70 EV), *m*/*z* (%): 258 (M^+, 76), 243 (60), 157 (100), 185 (91), 169 (65). 1-Ethyl-1,2,3,4-tetrahydro-β-carboline (ETC) was synthesized from tryptamine and propionaldehyde (Herraiz, 1997) (99% purity by HPLC). MS (70 EV), *m*/*z* (%): 200 (M^+, 15%), 171 (100). ^{1}H-NMR, ^{13}C-NMR, and GC–MS (N-methoxycarbonyl derivatives) (Herraiz, 1997) of synthesized compounds gave consistent spectra.

2.2. MTCA-EE in alcoholic beverages

Alcoholic beverages (Table 1), both from local and imported origin, were purchased in local supermarkets. Aliquots of each alcoholic beverage (50 ml) were spiked with 1 ml of a 5.3 mg/l solution of MTCA-ME as an internal standard (IS), adjusted to pH 9.1–9.5 and immediately extracted twice with 100 ml of dichloromethane using an extraction funnel. The organic phase was evaporated (30°C) under vacuum using a rotoevaporator down to ca 5 ml, treated with 0.1 M HCl (5 ml), mixed and evaporated again to remove CH_2Cl_2. The acidic extract was subjected to solid phase extraction using SCX columns (benzenesulphonic acid) (Bond Elut Varian, Harbor City, CA). The SCX columns (500 mg, 2.8 ml) placed on a vacuum manifold were conditioned with methanol (6 ml) and 0.1 M HCl (6 ml). The samples were passed through the SCX columns and washed with 0.1 M HCl (6 ml), 2 ml of methanol and 6 ml of milli-q deionised water. After rinsing with 2 ml of 0.4 M phosphate bufffer (pH 9.1), the tetrahydro-β-carboline ethyl esters were eluted using a 5 ml mixture of 0.4 M phosphate buffer: methanol (1:1), (pH 10.1).

Table 1
Occurrence of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester (1*S*,3*S*, *cis*) and (1*R*,3*S*, *trans*) in alcoholic beverages

Samples	No. tested	*SS*-MTCA-EE (μg/l)			*RS*-MTCA-EE (μg/l)			Alcohol (%)
		Mean	Range	SD	Mean	Range	SD	
Wines (red + white)	22	34.9[a]	2.3–112	26.22	15.7[a]	1.07–49.95	11.6	10–13
White wines	10	22.5[a]	2.3–41.8	13.9	11.4	1.08–22.1	7.49	10–12
Red wines	12	45.23[a]	10.1-112	29.95	19.2	5.01–50.0	13.4	11.5–13
Sherry wines	9	150[a]	75.5–393	107.1	66.4[a]	34.1–140	35.4	15–18
Sparkling wines	8	71.6[a]	52.0–94.3	16.8	33.0[a]	23.3–46.4	8.72	11.5–12
Port wines	5	27.6	22.1–33.6	4.96	11.8	10.6–13.6	1.4	19
Beer	6	3.5[a]	nd–6.88[c]	2.48	1.67[a]	nd–3.44	1.12	4.7–12
Brandy	6	8.1[b]	nd–19.6		2.73[b]	nd–9.0		30–40
Whisky	3	nd	–	–	nd	–	–	40–43
Vermouth	2	0.94	nd–1.89		nd			16
Sake	2	22.8	8.3–37.2	20.4	11.1	4.78–17.5	8.98	15
Wine for cooking	1	124	–	–	54	–	–	15
Bottled grape must	2	nd	–	–	nd	–	–	–

[a] Significantly different ($p < 0.05$) among groups.
[b] Only two brandies contained detectable MTCA-EE.
[c] Undetectable amount (nd).

Finally, the sample was acidified with drops of conc HCl and injected into the RP-HPLC.

Any possible formation of artifacts during the isolation procedure was checked using synthetic samples containing standards of the possible direct precursors, L-tryptophan ethyl ester and MTCA. Thus, solutions of 20 mg/l of TRP-EE or 18.6 mg/l MTCA in 50 mM phosphate buffer pH 3, containing either 12 or 30% v/v ethanol were spiked with 106 μg/l of MTCA-ME as internal standard (IS) and isolated through the entire procedure (liquid-liquid extraction and SCX) and analyzed for MTCA-EE.

2.3. Preparation of N-methoxycarbonyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester (N-MC-MTCA-EE) derivatives

N-methoxycarbonyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl esters (N-MC-MTCA-EE) derivatives were prepared from the corresponding MTCA-EE by reaction with methyl chloroformate reagent ($ClCOOCH_3$) (Aldrich) (Bosin & Jarvis, 1985; Herraiz, 1996, 1997; Herraiz & Sanchez, 1997). Thus, aliquots (0.5 ml) of MTCA-EE standard or *liq-liq* extracted MTCA-EE dissolved in 0.1 HCl, were mixed with 0.5 ml of 1 M phosphate buffer pH 7, and 50 μl of methyl chloroformate The mixture was vortexed for 1 min and allowed to stand for 5 min. The samples were then treated with 0.25 ml saturated Na_2CO_3 and 50 μl of methyl chloroformate and immediately vortexed and allowed to stand for 10 min. The derivatised samples were extracted with CH_2Cl_2, concentrated down to a few μl and injected into the GC–MS. An aliquot was also evaporated to dryness and redissolved in 0.1 M HCl:methanol (1:1) for analysis by RP-HPLC.

2.4. Chromatographic and quantitative analysis

2.4.1. RP-HPLC analysis

Chromatographic analysis was performed using a 1050 high performance liquid chromatograph with a 1046A fluorescence detector and a 3365-Series II HP Chemstation (Hewlett–Packard, Santa Clara, CA). A 150×3.9 mm, 5 μm, Nova-pak C18 column (Waters) was used for HPLC separation. Fluorescence detection was carried out at 270 nm for excitation and 343 nm for emission. Chromatographic conditions were: 50 mM ammonium phosphate buffer adjusted to pH 3 with phosphoric acid (Eluent A); 20% of A in acetonitrile (Eluent B). Gradient: 0%B to 32%B in 8 min, then 90%B at 18 min, 100%B at 20 min. The flow rate was 1 ml/min, oven temperature 40°C, and the injection volume 20 μl. MTCAs, MTCA-EEs and N-MC-MTCA-EEs eluted around 6, 10 and 15 min, respectively.

Quantitative analysis of MTCA-EE in alcoholic beverages was calculated from calibration curves obtained with standard solutions of known concentration ranging from 0 to 300 μg/l of MTCA-EE (mainly *cis* diastereoisomer). These standards were carried through the entire isolation procedure. Calibration graphs were constructed by plotting the peak area ratios of MTCA-EE relative to MTCA-ME (IS) against their concentration ratio. The two diastereoisomers were considered to have the same fluorescence response for quantitative purposes.

Confirmation of the identity of MTCA-EE isolated from alcoholic beverages was accomplished by HPLC retention time and coinjection with authentic standards. In addition, excitation and emission spectra of the HPLC peaks were compared with those of MTCA-EE standards. To achieve that, eluting peaks at the same

retention time as authentic MTCA-EE standards were trapped in the fluorescence detector flow cell by stopping the solvent pump and excitation and emission spectra recorded. On the other hand, the alkaline hydrolysis (drops of 1 M NaOH added to isolated extracts, ca pH $\geqslant$ 11) was carried out to test the hydrolysis of MTCA-EE and subsequent increase of MTCA (free acids) as an additional proof for MTCA-EE occurrence in alcoholic beverages.

2.4.2. GC–MS analysis

GC–MS analysis of the *N*-methoxycarbonyl derivatives obtained by reaction with methyl chloroformate was performed as described previously (Herraiz, 1997; Herraiz & Sanchez, 1997) by using an HP G1800A GCD system (GC–MS), consisting of a gas chromatograph, an electron ionization mass detector and a computer data system for obtaining and recording electron ionization (EI) mass spectra. A 20 m×0.25 mm i.d. methyl silicone capillary column was used with He as a carrier and programming temperature from 180°C (2 min), 4°C/min to 245°C (10 min). Injection was in the split mode (1:100); injector temperature: 260°C; transfer line: 280°C, and the ionization mode was EI at 70 EV scanning from *m*/*z* 10 to 425 with MS data acquisition starting at 5 min.

2.5. Preliminary study on the origin of MTCA-EE in alcoholic beverages

Aliquots of sherry wine spiked with an aqueous solution of L-TRP-EE hydrochloride (1 mg/ml) to represent concentrations of 0 (control), 2.16, 4.33, 8.66 and 17.3 mg/l, were prepared in duplicate and kept at room temperature for 9 days. 1-Ethyl-1,2,3,4-tetrahydro-β-carboline was added as internal standard (IS), and subsequently injected without sample clean-up into the RP-HPLC to determine the MTCA-EE produced by chemical condensation between the acetaldehyde of sherry and L-tryptophan ethyl ester. On the other hand, MTCA solutions (ca 10 mg/l) in 50 mM phosphate buffer, pH 3, and ethanol (15 and 30%) were heated at 70°C (61 h), and subsequently analyzed to test possible formation of MTCA-EE by chemical esterification.

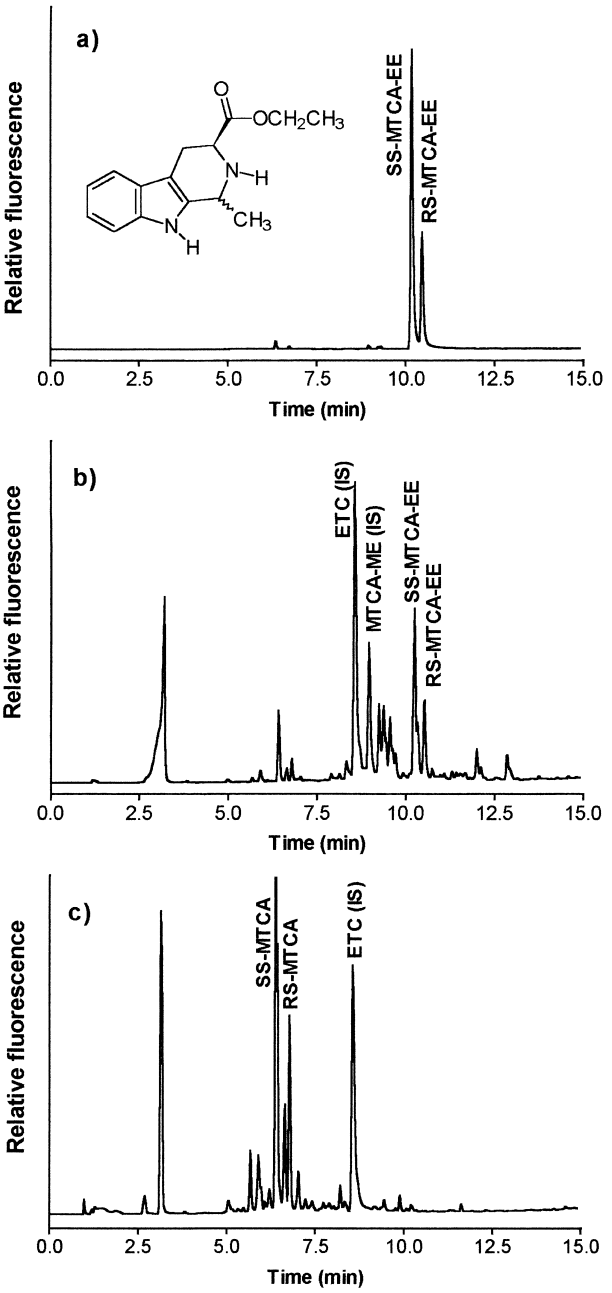

Fig. 1. RP-HPLC of MTCA-EE (1*S*,3*S* and 1*R*,3*S*) standards (a), MTCA-EE isolated from sherry wine (b), and MTCA-EE from sherry wine after alkaline hydrolysis (pH 11) (c).

3. Results

3.1. Chemical and chromatographic characterization of MTCA-EE in alcoholic beverages

Diastereoisomeric 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl esters (1*S*,3*S* and 1*R*,3*S* diastereoisomers) were well separated by RP-HPLC [Fig. 1(a)]. Tetrahydro-β-carboline-3-carboxylic acid ethyl esters isolated from wines gave peaks coeluting with authentic standards of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester [Fig. 1(b)]. Interestingly, the ethyl esters of tetrahydro-β-carboline-3-carboxylic acids were easily hydrolyzed under strong alkaline conditions (pH $\geqslant$ 11), giving rise to free acids (MTCA). This fact was useful to trace the presence of these compounds in real samples. Indeed, comparative results before and after alkaline hydrolysis of wine extracts strongly suggested the occurrence of these esters. Alkaline hydrolysis released free acid (MTCA,

1*S*,3*S* and 1*R*,3*S*) in both diastereoisomers while removing ethyl (MTCA-EE) and methyl (MTCA-ME) esters [Fig. 1(c)]. Moreover, the diastereoisomeric esters hydrolyzed to the corresponding acids without epimerization (results not shown).

A conclusive proof of the presence of MTCA-EE (1*S*,3*S*, 1*R*,3*S*) in alcoholic beverages was obtained by GC–MS of its *N*-methoxycarbonyl ethyl ester derivative following derivatization with methyl chloroformate ($ClCOOCH_3$) (Herraiz, 1997). MTCA-EE isolated from wine gave chromatographic peaks with consistent mass spectra of *N*-methoxycarbonyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester when reacted with methyl chloroformate (Fig. 2).

3.2. Occurrence of MTCA-EE in alcoholic beverages

Table 1 lists the amounts of MTCA-EE in alcoholic drinks. MTCA-EE, as sum of both diastereoisomers (1*S*,3*S* and 1*R*,3*S*) ranged from undetectable amount to 534 μg/l within the samples analyzed. The content of MTCA-EE was significantly different ($p < 0.05$) among groups of alcoholic drinks. Sherry wines contained the highest amounts (216 μg/l on average), followed by sparkling wines with 105 μg/l. Sweet fortified wines (called Ports) contained lower amounts than sherry wines (39.4 μg/l). Red and white wines had 64.4 and 33.9 μg/l on average, respectively whereas the sake analyzed contained 33.9 μg/l. Most distilled alcoholic beverages contain no MTCA-EE, although it was significantly present in two brandies (those brandies also contained MTCA). MTCA-EE was tentatively identified by HPLC retention time and alkaline hydrolysis in some high-alcohol beers but with much lower content than wines. As expected, no MTCA-EE was found in bottled grape must. MTCA-EE diastereoisomeric ratio *cis*/*trans* (1*S*,3*S*/1*R*,3*S*) ranged from 1.75 to 2.85, with an average of 2.21 ± 0.29 ($n = 48$). This ratio is lower than that of free acids (1*S*,3*S*-MTCA/1*R*,3*S*-MTCA) reported previously (Herraiz, 1996; Herraiz et al., 1993; Herraiz & Ough, 1993)

Following the excitation and emission spectra of HPLC peaks (trapped in the fluorescence detector cell), that correspond to MTCA-EE (both diastereoisomers) in real samples, it was confirmed that detected and quantified peaks corresponded to MTCA-EE. The fluorescence pattern (excitation and emission) of MTCA-EE in wines was very similar between diastereoisomers and very consistent with that of authentic MTCA-EE standards (Fig. 3).

The calculated recoveries of MTCA-EE and MTCA-ME (IS) from wines, considering the whole isolation procedure, were $93.2 \pm 1\%$ ($n = 4$) and $94.7 \pm 1.2\%$ ($n = 4$), respectively. The coefficient of variation (% RSD) for the repetitive analysis of MTCA-EE ($n = 4$, wine) was 1.8% on average; the reproducibility gave a

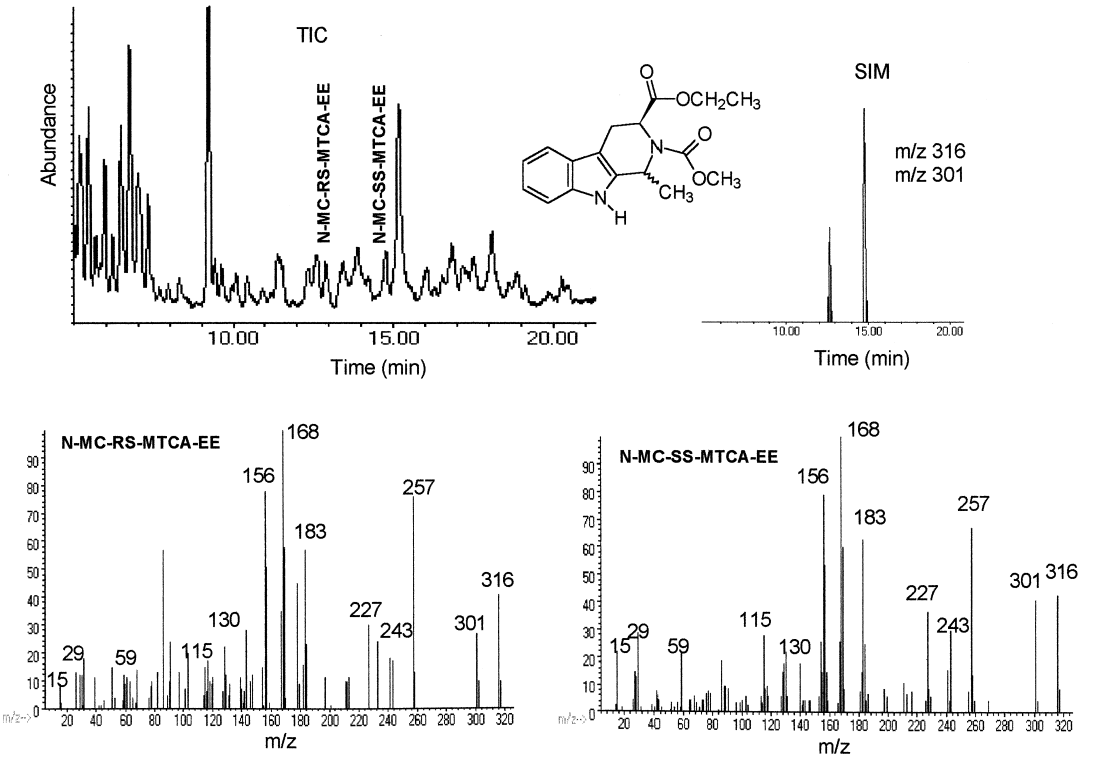

Fig. 2. GC–MS (total ion and selected ion chromatograms) and mass spectra of MTCA-EE (1*S*,3*S* and 1*R*,3*S*) isolated from wine, as *N*-methoxycarbonyl derivative.

RSD of 2% ($n=3$, sherry and sparkling wines), and the concentration was estimated with an error of less than 4% ($n=3$). Artifactual formation of MTCA-EE during the isolation procedure was ruled out since direct precursors of MTCA-EE, such as TRP-EE and MTCA (Scheme 1) in ethanolic solution (15 and 30% v/v ethanol, pH 3), subjected to the entire isolation procedure, did not give MTCA-EE. Neither were artifacts produced from MTCA-ME used as an internal standard.

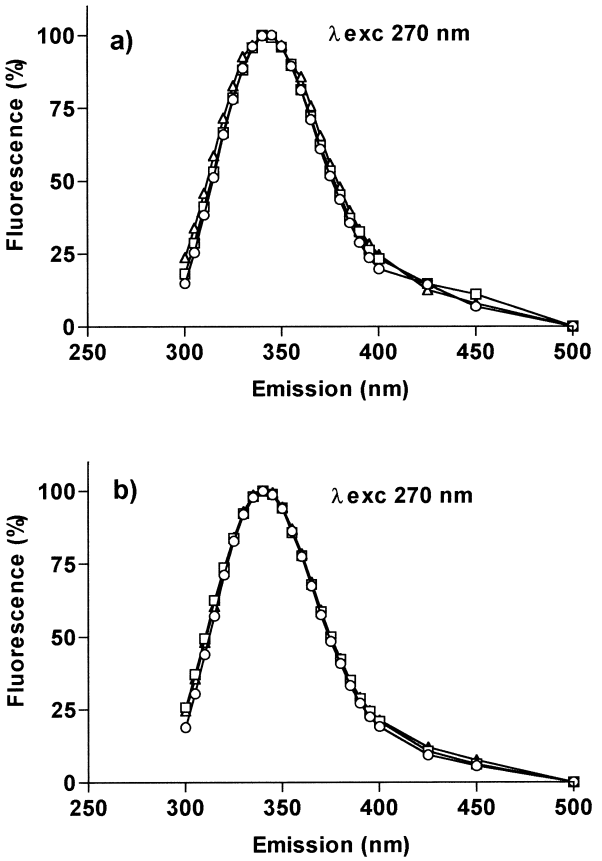

Fig. 3. Fluorescence emission spectra of *SS*-MTCA-EE (a) and *RS*-MTCA-EE (b) chromatographic peaks of authentic standards (○) and those from sparkling (□) and sherrry (△) wines. The emission spectra were obtained in duplicate when excitation was at 270 nm.

3.3. Preliminary studies on the formation of MTCA-EE

As illustrated in Scheme 1, the formation of MTCA-EE in alcoholic beverages may arise from free MTCA by esterification with ethanol or from L-TRP-EE through condensation with acetaldehyde. A slow chemical esterification from free MTCA might occur over time as observed by heating MTCA in the presence of ethanol (pH 3) (0.3% formed in 61 h). On the contrary, a good correlation was lacking among MTCA (free acid) (*SS* and *RS*) and MTCA-EE (esters) in alcoholic beverages as showed in Table 2. In contrast, the formation of MTCA-EE (both diastereoisomers) occurred readily from L-TRP-EE added to wine (Figs. 4 and 5). This suggests a rapid reaction between L-TRP-EE and the acetaldehyde occurring in sherry to give MTCA-EE. The same pattern occurred when L-TRP-EE and acetaldehyde standard solutions were reacted in acidic medium to give MTCA-EE in both diastereoisomers (data not shown).

4. Discussion

We have previously reported that fermented alcoholic beverages contain appreciable amounts of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA), reaching up to 0.65 and 17.5 mg/l, respectively (Herraiz et al., 1993). These carbolines arise from L-tryptophan and aldehydes released by yeasts during alcoholic fermentation, and their formation depends on the amount of precursors, storage time, pH, temperature and SO_2 content (Herraiz & Ough, 1993). Now, the research reported here shows the occurrence of ethyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (MTCA-EE) as two diastereoisomers (1*S*,3*S* and 1*R*,3*S*)

Table 2
1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (1*S*,3*S*-MTCA and 1*R*,3*S*-MTCA)[a] and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester (1*S*,3*S*-MTCA-EE and 1*R*,3*S*-MTCA-EE) in alcoholic drinks

Sample	*SS*-MTCA (mg/l)	*SS*-MTCA-EE (μg/l)	*RS*-MTCA (mg/l)	*RS*-MTCA-EE (μg/l)	Alcohol (%, v/v)
Red wine-1	4.57	125	1.4	51.3	12
Red wine-2	3.32	22.0	1.05	8.32	12.5
Red wine-3	4.83	58.10	1.53	22.0	13
Sherry wine-1	7.07	82.0	2.18	32.2	18
Sherry wine-2	6.78	122	2.11	53.4	18
Port wine	5.42	17.4	1.70	6.87	19
Sparkling wine-1	4.4	53.8	1.25	20.5	12
Sparkling wine-2	6.43	96.5	1.73	47.1	12
Beer-1	4.29	2.92	1.22	1.33	11
Beer-2	2.93	1.56	0.95	0.77	12

[a] MTCA was determined as previously (Herraiz, 1996; Herraiz et al., 1993).

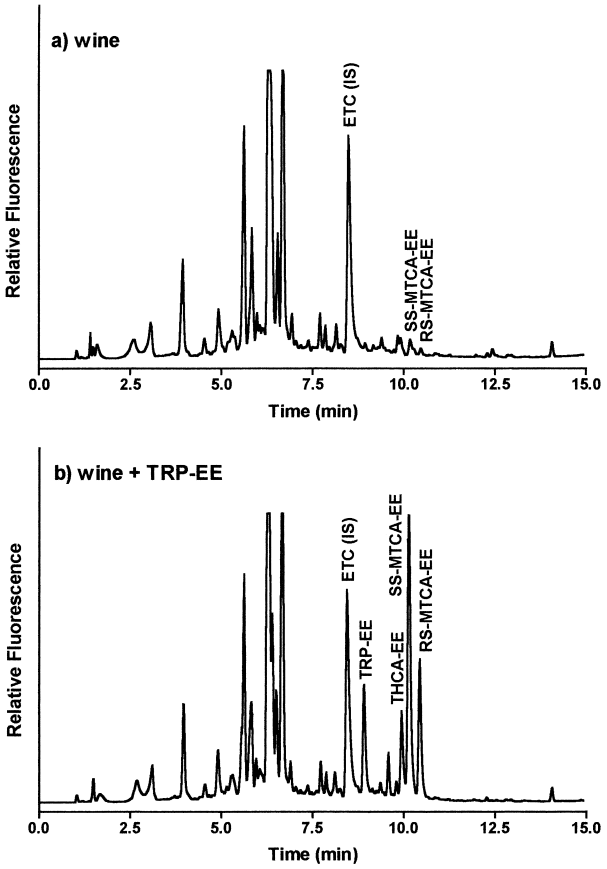

Fig. 4. MTCA-EE (1*S*,3*S* and 1*R*,3*S*) in sherry wine (a) and in sherry wine treated with TRP-EE (17.3 mg/l) (b). Samples were kept 9 days at room temperature and analyzed by RP-HPLC.

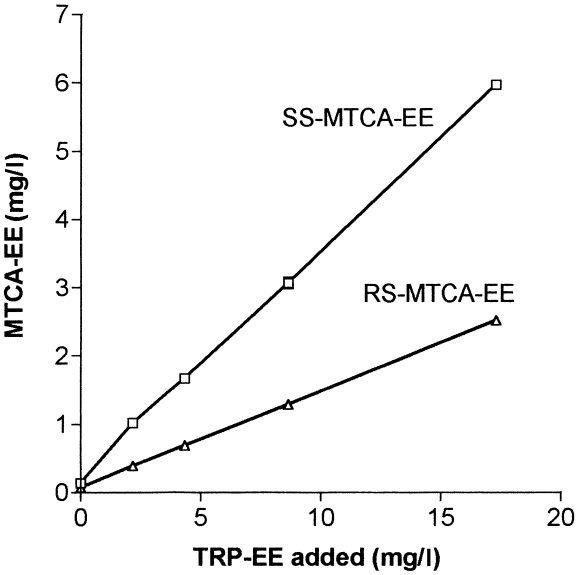

Fig. 5. Formation of MTCA-EE (1*S*,3*S* and 1*R*,3*S*) in sherry wine treated with increasing concentrations of TRP-EE. Samples were kept 9 days at room temperature. Points are averages of duplicates.

in fermented alcoholic beverages. It is the first report on β-carboline esters in those products. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid ethyl ester (THCA-EE), the corresponding formaldehyde-derived compound, was not unequivocally found in alcoholic drinks (at least at μg/l levels). Nevertheless, its presence in very low amounts could have been masked since THCA may occur in alcoholic beverages, and THCA-EE appears to form when TRP-EE is added to wine (sherry). The origin of MTCA-EE is unknown. A plausible hypothesis is the formation of MTCA-EE from tryptophan ethyl ester (TRP-EE) by chemical condensation and cyclization with acetaldehyde similarly to that reported from L-TRP and acetaldehyde (Herraiz et al., 1993; Herraiz & Ough, 1993).

The biological significance of tetrahydro-β-carbolines and β-carbolines is related to their pharmacological actions on the central nervous system, playing a role as neuromodulators (Buckholtz, 1980; Melchior & Collins, 1982; Myers, 1989; Rommelspacher et al., 1991). They have been involved in alcoholism (Adachi et al., 1993; Adell & Myers, 1994; Beck et al., 1982; Melchior & Collins, 1982; Myers & Melchior, 1977; Myers, 1989; Tuomisto et al., 1982), and in neurodegenerative disorders (Albores et al., 1990; Collins & Neafsey, 1985). Some of them, like MTCA or harmane, have been considered as precursors of mutagens, comutagens or simply toxic compounds (Brenneman et al., 1993; Higashimoto et al., 1996; de Meester, 1995; Wakabayashi et al., 1983). Although the concentration of MTCA-EE is not particularly high compared with that of the free acid MTCA, the ingestion of fermented alcoholic beverages would increase both MTCA and MTCA-EE in the body. These compounds, exogenously supplied (alcoholic drinks) or hypothetically produced in vivo (from acetaldehyde after ingestion of ethanol), might become bioactive, exhibiting behavioural and toxicological effects. In this regard, a hypothesis is that MTCA-EE could be oxidized to ethyl 1-methyl-β-carboline-3-carboxylate, that is a weaker analogue of β-carboline-3-carboxylic acid ethyl ester (β-CEE), a benzodiazepine receptor inverse agonist (Braestrup et al., 1980; Cain et al., 1982; Ninan et al., 1982; Tenen & Hirsch, 1980). Moreover, MTCA-EE from alcoholic beverages might not represent the sole source of this compound, and its possible formation in vivo following ingestion of ethanol might also be considered, as done for other THβCs.

5. Conclusion

A novel tetrahydro-β-carboline, chemically characterized as 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester (MTCA-EE), occurs in alcoholic beverages as two diastereoisomers (1*S*,3*S* and

1*R*,3*S*) reaching up to 534 μg/l. 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) and L-tryptophan ethyl ester (TRP-EE) are possible precursors leading to MTCA-EE. Interestingly, MTCA-EE is a reduced structural analogue (pyrido ring) of ethyl β-carboline-3-carboxylate (βCCE) that shows strong binding affinity to the benzodiazepine receptor.

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