analysis of the sensorially significant GPC fractions is needed to interpret the significance of the minor differences in the flavor notes between the two juices.

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Ethylcarbamate in Fermented Beverages and Foods. I. Naturally Occurring Ethylcarbamate

Cornelius S. Ough

A method was developed for the determination of ethylcarbamate in food and beverages. The ethylcarbamate was extracted, purified on Florisil, and concentrated. Separation was done with a specially prepared liquid phase. Detection was with a Coulson nitrogen detector. Most fermented foods and beverages measured contained ethylcarbamate, ranging from a trace to 6.0 μg/l. A commercial sake was found to contain in excess of 150 μg/l. Naturally occurring ethylcarbamate in wines was confirmed by gas–liquid chromatography and mass spectral analysis. The source was postulated to be the ethanolysis of carbamyl phosphate. No ethylcarbamate was found in unfermented foods or beverages.

Boehm and Mehta (1938), who first synthesized diethyl dicarbonate (DEDC), commonly called diethyl pyrocarbonate (DEPC), suggested that reactions with the primary amines formed the carbamic esters:

$$RNH_2 + O(OCOR')_2 \rightarrow RNHCOOR' + R'OH$$

The specific reaction of DEDC with ammonia in wine was mentioned by Thoukis et al. (1962). A report by Lofroth and Gejvall (1971) was the first on the amount of ethylcarbamate produced by adding DEDC to beverages. They reported finding large amounts by a radioactive isotope dilution determination. In a white wine of pH 3.4 with an estimated ammonia content of 5 mg/l., the addition of DEDC at 500 mg/l. apparently produced ethylcarbamate at 2.6 mg/l. That work was immediately repeated by others: Fischer (1971–1972) and Industrial Bio-Test Laboratories, Inc. (Department of Health, Education and Welfare, 1972), who found a much lower level (by a factor of about 100-fold) of ethylcarbamate formed by their isotope dilution studies. The possibility of ambiguity in the Lofroth work was strongly suggested. The second report also covered work concerning gas chromatographic determination of ethylcarbamate by conventional methods. Flame ionization techniques and gas chromatography combined with mass spectrometry could not verify the extremely high results. Those studies indicated levels 100 to 200 times less than that reported by Lofroth and Gejvall (1971). Nevertheless, permission for the use of DEDC in beverages under the Federal Food, Drug and Cosmetic Act (*Fed. Regist.*, 1972) was rescinded. The reason given was that deficiencies in analytical methods did not permit an unequivocal conclusion as to the presence or absence of ethylcarbamate in either treated or untreated beverages. Walker et al. (1974) recently reported a method capable of detecting ethylcarbamate in fermented beverages at 100 μg/l. The Joint FAO/WHO Expert Committee on Food Additives (World Health Organization, 1972) concluded that ethylcarbamate at 10 μg/l. was a permissible level in soft drinks, that the compound should be used only in beverages at pH 4.0 or less, and that diethyl dicarbonate not be used in beverages such as milk with significant ammonia, protein, and amino acids. The Committee felt its use in other acid beverages is not technologically justified and questioned whether ethylcarbamate naturally existed in fermented beverages. A number of questions thus appeared unanswered as to the amounts of ethylcarbamate that might occur naturally in fermented foods and the levels that could actually result from added DEDC. A series of experiments was initiated to resolve those questions. The first group of experiments was designed to detect the presence of naturally occurring ethylcarbamate and to explain the mechanism of its formation.

MATERIALS AND METHODS

Apparatus. A 1-l. Kuderna-Danish evaporator with a

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Table I. Recovery of Various Amounts of Ethylcarbamate Added to Wine

Wine type	Ethylcarbamate, μg/l. Added	Recovd	%[a]
White table	0	3.0	
	10	6.0	46.0
	10	11.0	84.5
	20	20.0	87.0
Red table	0	3.1	
	10	5.8	44.5
	25	20.0	71.5
	50	29.0	55.0
	75	60.0	77.0
			Av 66.5

[a] Corrected for original amount.

15-ml concentrating tube and 3-ball Snyder reflux condenser, a 10 × 300 mm chromatography column, and a Kontes microevaporator heater were used.

The gas–liquid chromatography oven and injector was a Varian 1700, and the columns were 6 ft × 0.25 in. glass, 4 mm i.d. Several different liquid phases were tested for separation of the nitrogen components: A = Versamide 3% + Ionox 220 0.5% on Chromosorb G 60–80 acid washed; B = 6 parts A + 9 parts (OV-17 10% + Carbowax 1500 5% on Chromosorb G 60–80 acid washed + 1 part (OV-1 3% on Chromosorb W); and C = OV-17 10% + Carbowax 1540 5% on Gas Chrom Q 100–120. The general retention times of ethylcarbamate on these columns are A, 3.4–4.0 min at 110 °C; B, 9–10 min at 125 °C; and C, 3.5–3.6 min at 130 °C. The flow rates were 30 ml/min of He. The injector temperature was 200 °C.

The detector was a Tracor Coulson in the nitrogen mode with a Hewlett-Packard 7127A recorder. The detector block was set at 220 °C, the pyrolysis oven at 820–860 °C, and Coulson sensitivity to 30 V with one or two times attenuation setting. Electrophoresis was done with a Camag 5000-V unit designed for use with paper. A Hewlett-Packard GC–mass spectrometer Model 5930A with Model 5933A data system was used. A 500 ft × 0.03 in. stainless steel OV-17 column was used at 150 °C with a retention time for ethylcarbamate of 13.3 min; or a 6 ft × 1.8 mm i.d. glass PPE-20 column on Haloport F at 110 °C with a 2.5-min ethylcarbamate retention time was used. Data were obtained for electron ionization (OV-17) at 70 eV and chemical ionization (PPE-20) as total ion gas chromatograms, limited mass (62 and 89) chromatograms, and mass spectra for single scan and for combinations summed and with background subtracted.

Thin-layer chromatography was done on 20 × 20 cm glass plates coated with silica gel G (no binder) to 300 μm thickness. The plates were activated for 1 h at 120 °C prior to use. The solvents were ethyl ether and petroleum ether (1:2).

Reagents used included: chloroform, redistilled with the first and last 5% discarded; benzene; diethyl ether; methanol; Florisil (as received from supplier); ethylcarbamate; ethyl acetate, redistilled with the first and last 5% discarded; carbamyl phosphate; petroleum ether; and 4-dimethylaminobenzaldehyde.

Procedures. The method used follows that of Walker et al. (1974) with some modifications to increase sensitivity. The ethylcarbamate additions and the percent recovery are given in Table I.

Special care was taken to use only glass or Teflon materials for the extractions. The glassware was degreased with hot 50% KOH or some suitable substitute. After degreasing and dichromate cleaning no beading should occur on the glassware. The ebulliators were cleaned and dried thoroughly prior to use.

The liquid beverages were used directly (except for the milk). Solid foods, 100–200 g, were mixed with excess (1 l.) water in a blender. The water phase was separated and reextracted two times more with 500 ml of H_2O. Combined water extracts were reduced in volume in a rotary vacuum flask to about 250 ml. For bread and milk, enough HCl was added to bring to 1 N concentration, and the mixture was brought to a boil and filtered. The chloroform extraction was done.

In 250 ml of the sample containing ethylcarbamate at 2 to 200 μl/l., 55 g of NaCl was dissolved and the sample then transferred into a 500-ml separatory funnel and extracted with 200 ml of chloroform (redistilled). It was mixed adequately to form a medium emulsion to assure good extraction and allowed to stand and separate. Any remaining emulsion was centrifuged. The sample was reextracted twice, with 100 ml of chloroform used each time. The extracts were combined and passed through about 50 g of anhydrous Na_2SO_4 held by a No. 1 Whatman filter paper and collected in a 1-l. Kuderna-Danish evaporating flask with a 15-ml bottom tube. The Na_2SO_4 was rinsed with 50 ml of chloroform and added to the rest. A few glass beads were added to the evaporator to prevent bumping, and a 3-ball Snyder reflux column was connected to the top of the evaporator. The unit was put on a steam bath and reduced in volume to 10 ml in a minimum time of 30 min. The flask was not allowed to go dry since ethylcarbamate sublimes and will readily be lost. The Snyder column and evaporator were rinsed with two 1.5-ml portions of chloroform, and the bottom tube was removed.

A Florisil column was prepared by first deaerating a slurry of Florisil in chloroform under vacuum. The slurry was then added to a 10 ml × 300 mm column (filled with chloroform), preventing air contact with the Florisil. The column was filled to a depth of 130 mm. Sufficient anhydrous Na_2SO_4 was added to cover the Florisil to a depth of 15 mm. The column was rinsed with 50 ml of chloroform, leaving a few milliliters covering the surface. The 12 to 13 ml of extract was added and slowly drained down until just the surface was covered. The tube was rinsed twice with chloroform, each time applying about 1.5 ml with a Pasteur pipet. The rinse was transferred onto the column. The column was rinsed down carefully with another 1.5 or 2 ml of chloroform. The ethylcarbamate was eluted from the column with 135 ml of a mixture of benzene, diethyl ether, and methanol (108:32:1). The first 10 or 12 ml (change in drop size denotes when chloroform has cleared the column) was discarded, and the remaining eluate was collected in a 1-l. Kuderna-Danish evaporator fitted with a 15-ml bottom tube and a few glass beads. Then 50 ml of ethyl acetate was added, and a 3-ball Snyder reflux column was placed on top of the Kuderna-Danish evaporator and the volume reduced in a steam bath to about 5 to 10 ml in 45 min. The residue was transferred with the adequate rinsings to the Kontes micro evaporator tube with ebulliator and a Kontes microevaporator heater, and a three-ring modified Snyder reflux condenser was used to reduce volume to 2.5 ml. The tube was removed from the heater and cooled, and the condenser was rinsed down and removed. The ebulliator was taken out and rinsed off into the tube. A Pasteur pipet was used to blow a gentle stream of N_2 onto the surface, and the volume was reduced to 0.5 ml at room temperature. The N_2 flow rate was about 5 ml/min, slow enough that the surface of the sample was not distorted by the flow.

Two to five microliters of the sample was injected into

Table II. Natural Ethylcarbamate Found in Some Fermented Foods and Beverages

Food or beverage	Source	μg/l. (or μg/kg) for column used A	B	C
Ale, U.S.	Grocery store	[a]	3.9	3.5
Beer, U.S.	Grocery store	0.6	0.6	0.5
Beer, German	Grocery store	1.8	1.2	0.6
Bread	Grocery store	2.2	1.2	1.0
Olives	Grocery store	0.8	1.2	1.1
Sake[b]	Liquor store	192	170 154	160
Soy sauce	Grocery store	4.8	3.9	
Yogurt	Grocery store	0.8	1.2	1.1
Wine (variety)				
Pinot noir	Univ. of Calif.	3.7	4.3[c]	
Ruby Cabernet	Univ. of Calif.	3.1	1.2[c]	
French Colombard	Univ. of Calif.	3.9	5.8[c]	
Malbec	Univ. of Calif.	4.4	2.2[c]	
	Univ. of Calif.	4.9	3.7[c]	
Barbera	Univ. of Calif.	4.0	2.9[c]	
Chardonnay	Univ. of Calif.	4.1	2.2[c]	
Cabernet Sauvignon	Univ. of Calif.	2.9	2.9[c]	
French Colombard	Univ. of Calif.	1.6	2.4[c]	
White Riesling	Univ. of Calif.	1.3	2.6[c]	
Thompson Seedless	Univ. of Calif.	2.7	2.3[c]	
Red Blend	Univ. of Calif.	3.6	1.5[c]	

[a] Large interfering peak not separated. [b] The diethyl carbonate residue in this sake was 0 mg/l. The pH was 4.24 and NH_3 = 52 mg/l. [c] These extracts were held in deep freeze several months prior to using with this column. (Values may be slightly high because of some volume losses.)

the GLC. Peak heights and retention time were measured. External standards were injected between every few samples to monitor changes in instrument sensitivity. Recovery curves were established by extracting added ethylcarbamate (Aldrich Chemical Co.) from the wine. Table I gives these data. Samples of freshly synthesized ethylcarbamate, which were chromatographed and collected, were compared with the commercial sample for purity by ir analysis. Amounts were calculated on the basis of ratio of peak height sample to peak height external standard × amount of external standard added × factor for recovery (based on average recovery data of Table I) × dilution factors.

Experimental. A large wine lot was used to determine whether any ethylcarbamate was naturally present in fermented beverages. Fifty-seven liters of a red wine of known history was selected, and the approximate ethylcarbamate concentration was determined. The volume was reduced by 20% by distillation; then NaCl was added and the sample extracted with chloroform. The extract was concentrated and passed through a Florisil column (all as previously described). The final concentrate was 10 ml. This was made basic, sparged with N_2, and then steam-distilled until 1000 ml had been collected (approximately 10% loss of ethylcarbamate). The distillate was saturated with salt, extracted three times each time with 1000 ml of ethyl acetate, and concentrated in a Kuderna-Danish evaporator to a final volume of a few milliliters. It was transferred to a Kontes microevaporator and concentrated to a few milliliters and reduced to 1 ml with a nitrogen gas stream. One milliliter was extracted with an equal volume of petroleum ether, and the petroleum ether fraction was discarded. The residue was extracted three times, each time with 1 ml of acidified water (pH 2.7). The water extract was neutralized and extracted three times, each time with 1 ml of ethyl acetate. The ethyl acetate extract was concentrated to 200 to 300 μl at room temperature in a micro test tube. The final

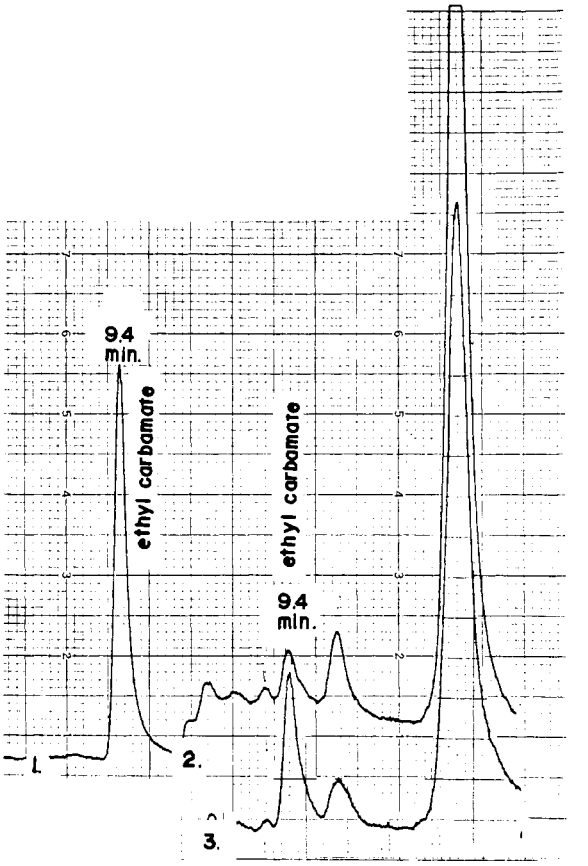


Figure 1. The response from Coulson detector for ethylcarbamate standard (1), a wine extract, untreated with diethyl pyrocarbonate (2), and sample 2 fortified with small amount of sample 1 to give sample 3; column B at 120° C and 16 psig of He.

concentration was between 20 and 100 mg/l. A few microliters of the concentrate was spotted on electrophoresis paper with known ethylcarbamate standards spotted on either side as markers. The voltage was set at 5000 V for about 30 min using pH 3.5 buffer. The paper was dried and the marker areas sprayed with 4-dimethylaminobenzaldehyde in acidified alcoholic solution to detect ethylcarbamate (Knappe and Rohdewald, 1966). The area where natural ethylcarbamate should be (moves about 3.5 cm) was cut out, extracted with ethyl acetate, and concentrated, and the presence of ethylcarbamate was determined with the Coulson detector. Areas of paper containing an unsprayed marker and one containing no treatment were extracted and concentrated, and no false positive peak resulted. The area cut out containing the suspect natural ethylcarbamate gave a positive result.

The results (Table II) show the concentrations of natural ethylcarbamate in various fermented foods and beverages as determined on the three different columns. The most efficient column was B. Figure 1 is an example of a wine chromatogram from the Coulson nitrogen detector.

Table III summarizes the results of the treatment of wine, beer, and bread with heat and 1 N HCl. Certain breads showed a very large increase in the peak area identified with ethylcarbamate when the whole bread was heated under acid conditions prior to extraction. Wine and beer had a twofold to threefold increase in extractable ethylcarbamate, whereas bread had increases of fourfold to about 40-fold.

No natural ethylcarbamate could be detected in any unfermented foods when concentrated extracts were investigated with the B column (Table IV).

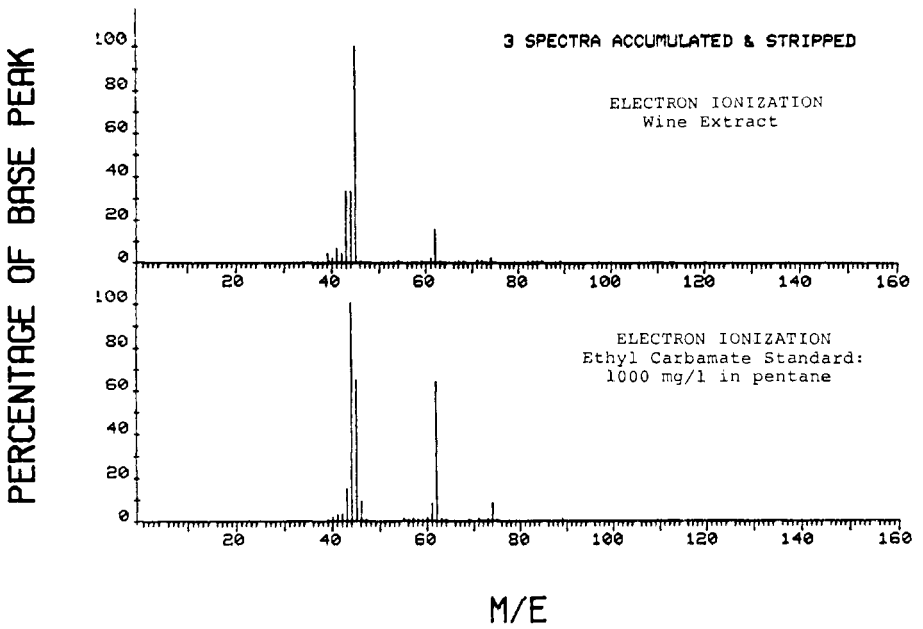


Figure 2. Comparison of mass spectra of ethylcarbamate and of ethylcarbamate from an extract from wine, using electron ionization.

Table III. Effect of HCl-Heat Hydrolysis of Bread, Wine, and Beer on Natural Ethylcarbamate Content

Product	μg/l. or μg/kg for column used					
	A		B		C	
	After[a]	Before[b]	After	Before	After	Before
Wine (white table)	2.6	5.3	1.3	2.4		
Beer (standard U.S.)	0.6	2.0	0.6	1.6		
Bread (white standard)	0.9	25	0.8	37	0.7	28
(white standard)	0.9	50	0.8	34	1.5	61
(whole wheat[c] std.)	1.4	6	1.6	3	1.5	10
(Fr. Sourdough)	2.4	23	1.7	17	1.6	21

[a] Refers to HCl-heat hydrolysis of the bread after the initial H_2O extraction. With wine and beer there was no "after" hydrolysis treatment but just a chloroform extraction. [b] Refers to HCl-heat hydrolysis before the initial H_2O extraction, or with wine and beer before the initial chloroform extraction. [c] These after bread sample and before samples were of similar type but different manufacturer.

Absolute Verification. The material suspected of being ethylcarbamate in the preceding experiment was examined by gas chromatography and mass spectrometry. The electron ionization mass spectrum exhibits the characteristic mass spectral features of ethylcarbamate, including a prominent 62-amu odd-electron fragment ion and 89 small-amu molecular ion (verified by limited mass chromatograms). The spectrum shows some distortion due to the small concentration of the compound in the sample, but the concentration is sufficient to result in a total ion peak at the retention time for ethylcarbamate on the OV-101 capillary column.

The chemical ionization mass spectrum also exhibits the characteristic mass spectral features of ethylcarbamate, including a prominent 62-amu odd-electron fragment ion and prominent 90-amu molecular ion + H^+ (verified by limited mass chromatograms). Retention time of the compound on the PPE-20 column is the same as that of ethylcarbamate.

GLC–mass spectrometry analysis of the 57-l. wine sample prepared for this purpose shows a positive identification of natural ethylcarbamate by both electron ionization (Figure 2) and chemical ionization (Figure 3).

Table IV. Natural Ethylcarbamate Found in Some Unfermented Foods

Food	Source	μg/l. for column used		
		A	B	C
Homogenized milk, deproteinized first	Grocery store	[a]	0.0	
Skim milk, deproteinized first	Grocery store	2.8	0.0	
Orange juice	Grocery store			
Fresh		1.5	0.0	2.7
Frozen concentrate		2.0	0.0	0.0
Grape juice, Welch's	Grocery store	0.6	0.0	0.0
Grape juice	Univ. of Calif.			
Early Burgundy		3.6	0.0	0.0
White Riesling			0.0	0.0
Pinot blanc			0.0	0.0
Animal food	Feed store			
Alfalfa (pressed cubes)			0.0	
Alfalfa (pellets)			0.0	

[a] Large interfering peak.

The amount of ethylcarbamate determined in the concentrated sample by chemical ionization technique is estimated at 10 to 30 mg/l. The original concentration of ethylcarbamate in the wine was 2.0 μg/l. Errors in small-volume estimations and in techniques can well account for the difference between these values and those obtained during the purification and concentration.

A large number of fermented foods and beverages were investigated by the GLC–Coulson nitrogen detector method; all had a detectable nitrogen compound at the proper retention time on either two or three different columns, with about the same quantity on each. These data are not conclusive but are strongly indicative of the presence of ethylcarbamate in most fermented foods. No ethylcarbamate could be detected in unfermented foods. The results of the heat–acid hydrolysis indicate the possibility that hydrolysable bound ethylcarbamate may be present in fermented food. The possible effect of stomach enzymatic digestion on the release of ethylcarbamate should be considered.

The high sake value did not come from the addition of DEDC to the finished beverage since no diethyl carbonate

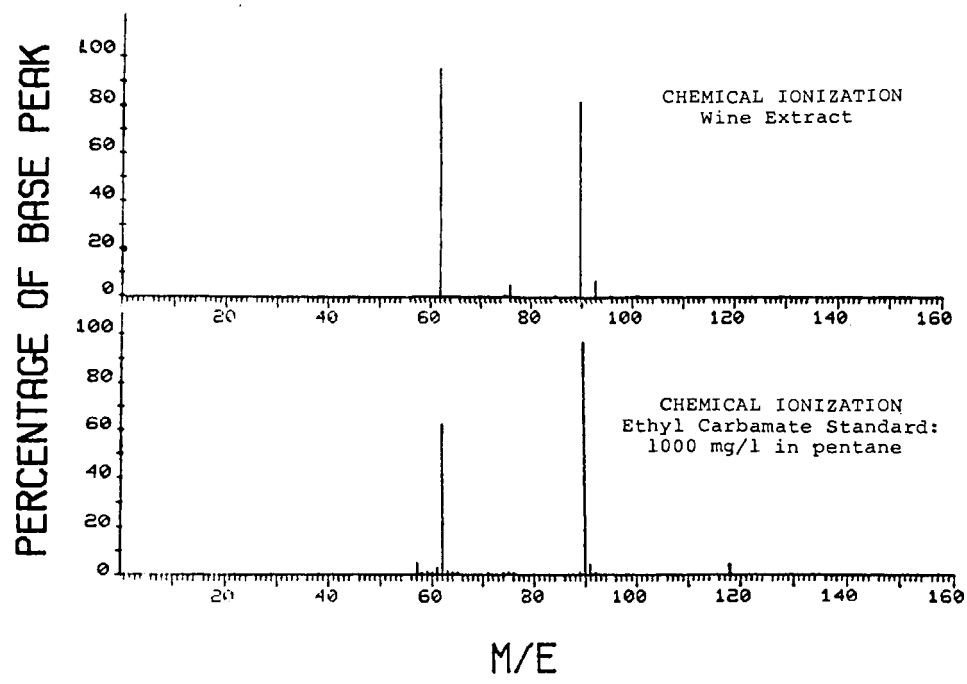
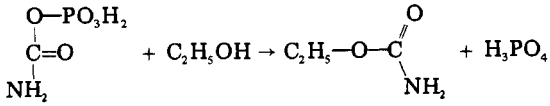


Figure 3. Comparison of mass spectrum of ethylcarbamate and of ethylcarbamate from an extract from wine, using chemical ionization.

was formed by reaction of DEDC with ethanol. It could have been formed by addition of DEDC to material prior to fermentation to sterilize the mash.

The source of natural ethylcarbamate seems likely to be the reaction of carbamyl phosphate with ethanol:



Halmann et al. (1962) and Allen and Jones (1964) discussed the hydrolysis of carbamyl phosphate. The reaction with ethanol would not be the preferred hydrolysis, though not precluded. Since small amounts of carbamic acid would be produced by water hydrolysis, small amounts of ethylcarbamate would be anticipated from ethanolysis. Williams et al. (1971) have shown discrete pools of carbamyl phosphate to exist in fungi at 3.5–6.0 mmol/g of dry weight and to increase tenfold under certain conditions. The carbamoyl-phosphate synthase (EC 2.7.2.5) that produces carbamyl phosphate from ATP, NH_3, and CO_2 in yeast is described by Barman (1969). The synthesis of carbamyl phosphate specifically in *Saccharomyces cerevisiae* is detailed by Lacroute et al. (1965).

Test for Chemical Formation of Ethylcarbamate. A sample of carbamyl phosphate (Nutritional Biochemical Corp.) was allowed to react with various amounts of ethanol at various concentrations in a pH 3.5 citric acid–phosphate buffer. After standing for 72 h at room temperature the samples were extracted twice with equal volumes of ethyl acetate, they were concentrated by the described method, and the amounts were determined by GLC–Coulson detector analysis. Certain samples were chromatographed on silica gel along with marker ethylcarbamate spots. The ethylcarbamate was identified by using a 4-dimethylbenzaldehyde spray with an R_f value of 0.40. A canary-yellow spot develops only if the spotted samples contain 5 μg or more.

The results of the reaction of carbamyl phosphate and ethyl alcohol (Table V) show ethylcarbamate forms. The positive thin-layer identification of ethylcarbamate is obtained with the highest levels of ethanol and carbamyl phosphate tested. The amount of ethylcarbamate produced is roughly proportional to the alcohol and to the carbamyl phosphate concentrations.

Table V. Ethylcarbamate Produced by Reaction of Ethyl Alcohol and Carbamyl Phosphate or Urea and Water Solutions at pH 3.50

Carbamyl phosphate,[a] g/l.	Urea added, g/l.	Ethanol, v/v %	Ethyl-carbamate, μg/l.
0.01	0	0.0	1[b]
0.01	0	2.75	15
0.01	0	5.5	36
0.01	0	11.0	99
0.10	0	11.0	1400
0.00	2.0	11.0	20

[a] The carbamyl phosphate contained urea as an impurity of 1 or 2% by weight. [b] Carbamyl phosphate impurity.

CONCLUSIONS

The presence of natural ethylcarbamate in wine has been positively demonstrated. Other fermented foods, including bread, probably contain as much or more. In addition, one probable natural source of this material has been identified in fermented beverages and other fermented foods. It appears to be ubiquitous in nature.

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Ethylcarbamate in Fermented Beverages and Foods. II. Possible Formation of Ethylcarbamate from Diethyl Dicarbonate Addition to Wine

Cornelius S. Ough

Determination was made of the effects on the formation of ethylcarbamate in model solutions that pH, ammonia, and diethyl dicarbonate (DEDC) have in the ranges found or used in acidic beverages. Prediction of the maximum possible amount of ethylcarbamate that can be formed is expressed graphically in terms of the three variables. DEDC added to wines forms less than the amounts predicted because of other competitive reactions. The amounts of ethylcarbamate formed in commercial wines, under controlled conditions, are less than 1 μg/l. The amounts formed under optimum industrial conditions are below the sensitivity of the test to detect. These data indicate that the compound's FDA status should be reevaluated.

A preceding article (Ough, 1976) discussed the recent history of diethyl dicarbonate (DEDC) and the reasons for its discontinuance as an additive to beverages and foods. The results reported in that paper clearly indicate that ethylcarbamate is a natural component in wine and is probably present in all fermented foods and beverages.

This article describes tests made on model solutions and on wines in which the parameters that affect the formation of ethylcarbamate when DEDC is added were controlled or known.

EXPERIMENTAL SECTION

Extraction, concentration, and analysis by gas chromatography with the Coulson detector have been described (Ough, 1976).

NH_3 analyses were performed by specific ion electrode techniques as described by McWilliam and Ough (1974).

Since the formation of ethylcarbamate from added DEDC would have to occur from its reaction with ammonia, an experiment was designed to determine the degree to which it could be formed in model systems without competing reactions. These levels would then represent the maximum obtainable with the pH, NH_3, and DEDC levels investigated.

pH–Ammonia–DEDC Parameters for Ethylcarbamate Formation. Buffer solutions were made with 0.2 M K_2HPO_4 and 0.1 M citric acid, both in 11% v/v ethanol. Precalculated amounts were mixed to give a series of solutions with pH values of 3.0, 3.25, 3.50, 3.75, and 4.00. Final adjustments were made with concentrated NaOH or H_2SO_4. Each pH solution was subdivided into four sublots, and the ammonia content of each was adjusted to 0, 50, 100, and 200 mg/l. Each of these samples was subdivided further into five lots, and DEDC (obtained from Bayer AG and checked for purity, exceeding 99.5%) was added at 0, 50, 100, 200, and 400 mg/l. Samples were stored for 72 h at 20 °C. The samples were analyzed by saturating 250 ml with 70 g of NaCl and extracted three times each with 100 ml of ethyl acetate; the extracts were combined, dried over Na_2SO_4, and reduced in volume as described previously (Ough, 1976). A standard curve was prepared. Peak responses of samples were compared with that of a similar external standard using a 3% Versamide liquid phase on Chromosorb G 60–80 acid washed (column A as described by Ough, 1976). Recovery from model solutions was consistent and in excess of 80%. Measurements of peak heights gave linear response over a wide range. The amounts of ethylcarbamate produced at various combinations were calculated from a standard curve and external standards.

The data (Table I) show the amounts of ethylcarbamate produced in the various combinations of variables. The data are further displayed in the plot of Figure 1 (log of the micrograms of ethylcarbamate produced/milliliter of ammonia present compared with the pH), indicating a linear response.

Since these data permitted prediction of the maximum amounts of ethylcarbamate that could be formed under the conditions described, a number of wine samples representative of a broad spectrum of commercial products were treated with various levels of DEDC and analyzed for any ethylcarbamate that might result.

Two hundred milligrams of DEDC (Bayer AG-purity 99.5%) was added per liter to a number of wines (prepared at the University of California Enology Laboratory). After 72 h or more at 20 °C NH_3 at about 100 mg/l. was added to samples of some of the same wines, and DEDC was also added. Wines were analyzed for original pH and NH_3 content.

These data are shown in Table II. In addition, analysis of the ethylcarbamate in these wines is reported and plotted in Figure 2. Measurement was as reported previously (Ough, 1976). The lower limit of detection is about 2.5 ng/injection (threefold to fivefold more peak height than baseline variation). The reproducibility of the method, measured on 19 replicated samples ranging from 1.6 to 5.2 μg/l., reported as relative standard deviation, is

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