molecules. The investigation of intramolecular reactions is of great importance because of their striking analogy with enzyme catalysis which proceeds through an enzyme–substrate complex. Imidazoles were the first organic bases whose catalytic role was evidenced in the hydrolysis of esters. Imidazole and its derivatives have been much studied because of the apparent involvement of the imidazolyl group of histidine at the active site of many hydrolytic enzymes such as chymotrypsin, trypsin, cholinesterase, or ribonuclease (Bruice and Benkovic, 1966).

Furthermore, the kinetic data reported above are of some practical value by allowing a better choice of solvents for analytical techniques (extraction, clean-up, chromatography, etc.) relative to benomyl determination.

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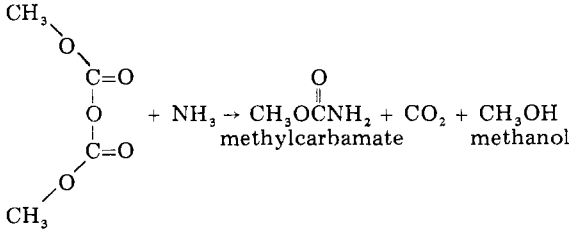
Measurement of Methylcarbamate Formed by the Addition of Dimethyl Dicarbonate to Model Solutions and to Wines

A method for ethylcarbamate analysis was adapted for detecting methylcarbamate formed from dimethyl dicarbonate added to solutions and wines containing varied amounts of ammonia and at different pH values. Recovery from wine by this method is 51%, less than found for ethylcarbamate in the original study. The effects of pH and NH_3 concentration on the amount of methylcarbamate formed are similar to those for ethylcarbamate. Under the most extreme conditions in normal commercial wine practices (pH $\leq$3.75, $NH_3 \leq 20$ mg/l.) less than 10 μg of methylcarbamate per l. would be formed from the addition of dimethyl dicarbonate at 100 mg/l.

Diethyl dicarbonate (DEDC) as an additive to wine has been discussed at some length in regard to the consequent formation of ethylcarbamate (Ough, 1976b). An additive which might be substituted for DEDC is the dimethyl dicarbonate (DMDC), a compound with similar fungicidal properties but resulting in methyl side products instead of ethyl compounds. The effectiveness of DMDC (Ough, 1975), the amount of methanol formed, and the methods of measuring it (Stafford and Ough, 1976) have been reported. The methylcarbamate is produced by the reaction of ammonia with the DMDC.



This work was done to determine whether the methylcarbamate forms in a manner similar to the formation of ethylcarbamate from DEDC and ammonia. Secondly, methods used to measure ethylcarbamate are applied to find how much methylcarbamate would be formed in wine.

METHODS AND MATERIALS

The equipment used was similar to that reported for measuring ethylcarbamate (Ough, 1976a). All the usual precautions were taken, as discussed previously. Pure methylcarbamate was obtained from Pfaltz and Bauer Inc. Experimental samples of DMDC were obtained from Logica International Corp. The samples were in excess of 99.5% pure by our analysis.

The ammonia content of the wines and buffer solutions was measured with a Beckman research pH meter and an Orion ammonia probe, Model 95-10.

The chloroform (Mallinckrodt, A.R.) and ethyl acetate (Mallinckrodt, A.R.) used were redistilled, and a 5% head and a 5% tails cut removed. All other reagents, reagent grade or better, were used as received. Solvent blanks showed no detectable peak at the retention time of methylcarbamate.

Total phenols and pH were determined as given by Amerine and Ough (1974).

Model solutions were prepared by combining appropriate volumes of 0.1 M dibasic potassium phosphate and 0.2 M citric acid, both in 11% v/v ethanol, and diluting to volume with 11% v/v ethanol (1 part buffers + 3 parts ethanol solution). Ammonia was added as $(NH_4)_2SO_4$, and final pH adjustments were made with concentrated NaOH or H_2SO_4. DMDC was added by the appropriate microliter syringe. Samples were allowed to stand at 20 °C for at least 24 h before extraction and analysis. The analytical procedure was similar to that reported for model solution and wine analysis for ethylcarbamate (Ough, 1976a). The model solutions were extracted with ethyl acetate. Extracts of model solutions were concentrated and analyzed directly. The chloroform extracts of the wines were purified on Florisil PR 60/100, and the eluate was concen-

Table I. Recovery of Added Methylcarbamate to Model Solutions and to Wines

Material	Concn, μg/l.	% recovery
Model solution	0	<2.0
Model solution	10	72.0
Model solution	20	60.0
Model solution	50	80.0
Model solution	100	66.0
		Av 69.5
Wine	10	45, 55
Wine	10	54
Wine	20	55, 55
Wine	40	44
Wine	80	52
		Av 51.0

trated and analyzed. The methylcarbamate was separated by gas–liquid chromatography. A Coulson detector in the nitrogen mode was used as the detector. Quantification was by peak height comparison with an external standard. Retention time was 6.1 min. The column used was similar to that reported for ethylcarbamate separation: 9 g of 10% OV-17 + 5% Carbowax 1500 coated together on Chromosorb G 60/80 AW, 6 g of 3% Versamide + 0.5% Ionox 220 coated together on Chromosorb G 60/80 AW, and 1 g of 3% OV-1 on Chromosorb W 60/80 AW, all mixed together and the column packed with the mixture. Operating conditions were 125 °C oven, 185 °C injector, 200 °C transfer lines, and 840 °C pyrolysis oven.

RESULTS

Table I shows the recoveries of the methylcarbamate added to model solutions and to wines. The simpler method for the model solution gave a better recovery, though less than the recoveries in the previous work (Ough, 1976b) for ethylcarbamate (80%). Likewise, the recoveries from the wines were less than the 65–70% recovery for ethylcarbamate.

The series of model solutions was analyzed. Figure 1 plots the amounts found. Increasing pH caused increased amounts of methylcarbamate to be formed. Increased ammonia also caused increased methylcarbamate to be formed. The pH (linear) vs. the micrograms of methylcarbamate per milligram of NH_3 (log function) plot as a straight line.

Table II presents the analytical data on the wines and the results of the treatment of the wine sample with DMDC. The amounts of methylcarbamate are in the same ranges suggested by the model solution work. The extremely high pH of the Tinta Madeira wine is unusual.

DISCUSSION

The poor extraction of the methylcarbamate from the polar solutions is the main reason for the low recovery. The methylcarbamate should be more soluble in the polar phase than was ethylcarbamate.

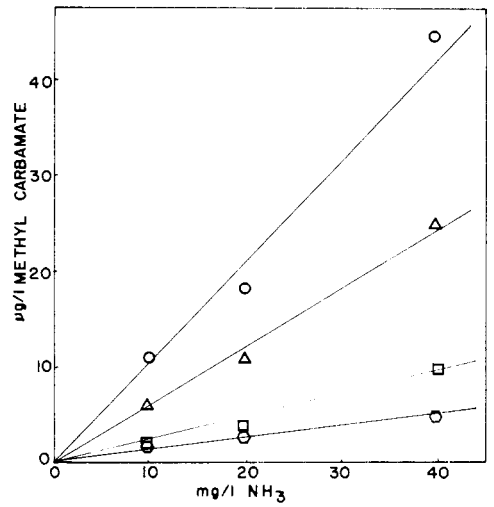


Figure 1. The relative effect of pH on the amounts of methylcarbamate formed by treatment of NH_3 in model solutions with dimethyl dicarbonate at 100 mg/l.: hexagons, pH 3.0; squares, pH 3.23; triangles, pH 3.67; and circles, pH 4.0.

The reactions of DMDC and DEDC with ammonia appear to be similar in extent. The comparative amounts of methyl- to ethylcarbamate formed both in model solution and in wines from DMDC and DEDC treatments are in agreement. A small chromatographic peak present at the retention time of methylcarbamate in the untreated wines may or may not be methylcarbamate. For the purposes of the calculations it was measured as methylcarbamate. The postulated natural formation route of ethylcarbamate (Ough, 1976a) in fermented beverages is the ethanolysis of carbamyl phosphate. Since wines naturally contain methanol at 20–150 mg/l., small amounts could conceivably be formed. However, considering the relatively small amounts of ethylcarbamate formed during this reaction and the methanol concentrations normal in wine, it is highly unlikely that this peak is methylcarbamate. The detectable limit by this method is about 0.5 mg/injection or 2 μg/l. original concentration since recovery is only about 50%. Therefore, if pH drops below 3.3, then DMDC treatment at 100 mg/l. gave amounts of methylcarbamate lower than the levels detectable in these wines. A number of other reactions are operative and in competition for the added DMDC. Among these are the reactions with the phenol compounds, organic acids as well as amino acids, amines, and other basic organic materials. The high phenol content of red wines (usually about tenfold that of white wine) could explain the relatively lower amounts of methylcarbamate formed in the red

Table II. Amounts of Methylcarbamate Formed in Wine by Addition of Dimethyl Dicarbonate[a]

Wine variety	Total phenols, mg/l.	pH	NH_3, mg/l.	Methylcarbamate, μg/l.		
				Control	Treatment	Net formed
White						
Sylvaner	191	3.41	3.0	2.4	5.1	2.7
French Colombard	226	3.34	5.0	4.1	5.9	1.8
Chenin blanc	297	3.24	2.3	2.0	2.0	0.0
White Riesling	282	2.99	1.4	2.0	2.0	0.0
Chenin blanc	68	2.86	1.2	2.0	2.0	0.0
White Riesling	53	2.87	1.6	2.0	2.0	0.0
Red						
Tinta Madeira	2290	4.40	8.7	5.5	12.9	7.4
Cabernet Franc	1840	4.05	5.1	4.7	6.5	1.8
Barbera	535	3.43	5.3	3.1	3.5	0.4
Malbec	1130	3.33	4.1	2.0	2.0	0.0

[a] DMDC added at 100 mg/l.

wines. It is suggested that the hydroxide groups would react with the DMDC and the combination would decrease the chance that the DMDC would react with ammonia to form the methylcarbamate. More data are required before that can be verified. Surprisingly little is known about these reactions with DMDC.

Methylcarbamate has been investigated for carcinogenic activity (Pound, 1967). The report states that it has none. The toxic level is reported at LDLd: 500 mg/kg taken intraperitoneally (U.S. Department of Health, Education and Welfare, 1973).

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Geosmin, the Earthy Component of Table Beet Odor

The volatile components of red table beets (*Beta vulgaris* L., C.V. Ruby Queen) were prepared by fractional steam distillation, solvent extraction, and concentration, and then separated into acid, base, and neutral fractions by further solvent extraction. The base fraction had a potato-like odor, the neutral part had a distinct earth-like odor, and the acid fraction was almost odorless. Analysis of the neutral fractions by gas chromatography and mass spectrometry yielded a single major earthy smelling component identified as geosmin, a C_{12} terpene-like compound known to be produced by soil organisms of the order Actinomycetes.

"Earthy" odors have been described in several vegetables and vegetable products as being due to pyrazine compounds (Deck and Chang, 1965; Parliment and Epstein, 1973; Buttery and Ling, 1973). However, the "earthy" or "musty" odor caused by the contamination of food and water supplies by Actinomycetes (Morris, 1962; Romano and Safferman, 1963; Silvey and Roach, 1964) is due to geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) identified by Gerber (1968), Marshall and Hochstetler (1968), and Kikuchi et al. (1972). This paper shows that geosmin is also a major component of beet essence and is responsible for this vegetable's characteristic "earthy" odor.

EXPERIMENTAL SECTION

Beet Juice. Red table beets (*Beta vulgaris* L., C.V. Ruby Queen) grown at this station during the 1973 season were graded for size (2.5 to 6.0 cm in diameter), washed, blanched in boiling water for 15 min, and cooled in running water. Beets (500 kg) were then ground in a Fitzpatrick hammermill, mixed with filter aid (KeyCel press aid, 1.5% w/w), and pressed in a hydraulic press, to yield 300 kg of beet juice.

Aqueous Essence. The beet juice was preheated to 100 °C by pumping it through a steam jacketed heat exchanger and then the hot juice was fed continuously to the top of a stainless steel 10 plate distillation column (Moyer and Saravacos, 1968). A clear colorless aqueous essence with a strong beet-like odor was obtained after two cycles through the column representing a 52-fold concentration.

Acid, Base, and Neutral Fractions. Three 300-ml portions of aqueous essence were extracted with equal volumes of Freon 113 (1,1,2-trichlorotrifluoroethane) and the fluorocarbon layer was concentrated at atmospheric pressure by distillation (2 cm o.d. by 90 cm Vigreux) to 30 ml. The concentrated extract was further extracted with 30 ml of 1 N HCl to remove the bases, followed by 30 ml of 1 N NaOH to remove the acids, and then concentrated to about 0.3 ml in a 10 plate microdistillation column to yield the neutral fraction. Both the aqueous acid and base layers were neutralized, back extracted with Freon 113, and concentrated to produce the base and acid fractions, respectively.

Gas Chromatography, Odor Evaluations, and Mass Spectrometry. An 80 m × 0.75 mm i.d. stainless steel open tubular gas chromatographic column coated according to the procedure of Mon (1971) with 5% SF 96 and 3% Triton X305 was used in a Varian Model 1440 gas chromatograph. It was operated isothermally at 60 °C for the first 3 min and then programmed at 2 °C/min for 50 min. Helium was used as a carrier gas (12 ml/min) and the output of the column was coupled to a time of flight mass spectrometer (Bendix Model 12 modified with a CVC Mark IV) through a methylsilicone helium separator (Black et al., 1969). Ionization was at 30 °C and 70 eV. The volatile components eluted from the gas chromatograph were detected by monitoring the total ion current of a mass spectrometer and simultaneously evaluating the odor by sniffing the output of the helium separator.

Synthesis of Geosmin. Geosmin was synthesized according to the procedure of Marshall and Hochstetler (1968). The resulting mixture of isomers (50 mg of an almost colorless oil) was separated in a 4 m × 3 mm gas chromatographic column containing Chromosorb W coated with 5% SP 1000 (a Carbowax modified with terephthalic acid, obtained from Sepelco Inc., State College, Pa.). The column was used in the gas chromatograph–mass spectrometer system described above and operated with a carrier gas flow rate of 20 ml/min and the oven temperature was programmed from 60 to 180 °C at 4 °C/min.

RESULTS AND DISCUSSION

The concentrated extract of beet essence described above has the characteristic odor of beets. However, the neutral fraction retained the earthy character of the whole