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Ethyl Nitrite. The *in vivo* Synthesis of a Mutagenic Substance from Tobacco Smoke and Ethanol

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Gas chromatography–mass spectrometry demonstrates the presence of ethyl nitrite in the breath of volunteer subjects who smoke and drink concurrently. The mutagenic compound is quantitated at the ppb level by gas chromatography with the selective electron capture detector. The compound is not detected in cases of only drinking or only smoking.

We report a similarity between the pollutants produced in the automotive combustion of alcohol/gasoline blends and those produced in a person who is smoking tobacco while drinking diluted alcohol. In using alcohol/gasoline blends, there results not only typical combustion products and unburned gasoline but also unburned alcohol.[1] Concomitant nitrogen oxides then react rapidly at room temperature with unburned alcohol to yield alkyl nitrites,[2,3] provided that the reactant concentrations are high enough. The stoichiometry of the reaction is shown below.

$$ROH + 2NO_2 \rightleftharpoons HNO_3 + R-O-N=O$$

Ethyl nitrite was shown to be strongly mutagenic in bacteria and barley.[4] This laboratory is actively investigating analytical methods for the determination of such alkyl nitrites in the environment. We postulated that in an incident of smoking tobacco while drinking ethanol, the body cavities could serve as a reaction vessel for the gas phase synthesis of ethyl nitrite (b.p. 16 °C). The nitrogen oxides produced at the temperature of burning tobacco (~800 °C) were expected to react rapidly with ethanol vapor in the gas phase in a body cavity (*e.g.* mouth, nose, trachea or lung) to yield ethyl nitrite, and this possibility was investigated.

EXPERIMENTAL

Ethyl nitrite (labeled concentration of 15 % in ethanol solution) was obtained from Merck-Schuchardt, Hohenbrunn bei München, G. F. R.. The composition of the solution was confirmed by gas chromatography–mass spectrometry (GC/MS) on an LKB Model 9000 modified for glass capillary columns. Column effluent was measured by MS total ion current. There were two main peaks that constituted 99 area percent of the chromatogram and there were no significant impurities. The electron impact mass spectrum at 70 eV of the smaller chromatographic peak is consistent with the literature spectrum of ethyl nitrite.[5] The mass/charge, likely fragments, and relative intensities of the main peaks are 30, NO^+, 1000; 29, $C_2H_5^+$, 245; 45, $C_2H_5O^+$, 141; 60, CH_2ONO^+, 308. Response factors were not available for quantitation by MS and the concentration of ethyl nitrite in the purchased ethanolic solution was determined on a JEOL Model JNM-PMX 60 nuclear magnetic resonance spectrometer. The calculated concentration of the ethyl nitrite was 8.3 % (W/W). The solution was then kept in the dark at −18 °C and removed only to develop the chromatographic procedures.

The gas chromatograph was fabricated in this laboratory and is equipped with a Packard electron capture detector (supplied with the Model 302 ECD linearizer) with 10 mCi ^{63}Ni radioactive source. Operating conditions are in the caption of Fig. 1. The limit of detection for ethyl nitrite under these

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Table 1. Summary of results. ND, not detected; NA, not analysed.

Experimental conditions[a]	Ethyl nitrite concentrations			
	Subject A		Subject B	
	ppb (v/v)	pg/500 μl	ppb (v/v)	pg/500 μl
a. Before drinking	ND	ND	ND	ND
b. Before smoking	ND	ND	ND	ND
c. Smoking, not drinking	ND	ND	ND	ND
d. Drinking, not smoking	ND	ND	ND	ND
e. One drink followed by inhaling smoke in lungs for				
i. 1 s	48	74	70	108
ii. 3–5 s	47	73	54	84
iii. 10 s	42	65	61	95
f. Keeping alcohol + smoke in mouth for 10 s	92	143	176	273
g. One drink swallowed and then smoking one cigarette				
i. immediately after	37	57	120	186
ii. 1.5 min after	NA	NA	18	28
iii. 5 min after	5	7	19	29

[a] The 500 μl gas-tight syringe was inserted into the subject's mouth for sampling and the sample injected immediately into the gas chromatograph.

operating conditions is one part in 10^9 (v/v) or 1.6 picograms/500 μl. The calibration plot is linear from this one ppb up to at least 500 ppb.

RESULTS

The volunteer subjects ingested 60 ml of 40 % (v/v) ethanol in water under varying experimental conditions of exposure to tobacco smoke. Experimental conditions and analytical results are shown in Table 1 and typical chromatograms for some of these data are shown in Fig. 1. The breaths of the subjects were sampled with a 500 μl gas-tight syringe and showed no detectable ethyl nitrite (less than 1.6 pg/500 μl) (a) prior to only smoking, (b) prior to only drinking, (c) after only smoking, (d) after only drinking. The data indicate that only when tobacco smoke and alcohol are contained together in the body cavity is ethyl nitrite produced. That the chromatographic peak from a test subject is indeed from ethyl nitrite was confirmed by identical retention times for sample and standard during additional gas chromatography with the selective electron capture detector on a stationary phase of different polarity, 6 % Fluorad FC-431 (3M Company, St. Paul, Minnesota, 55101, U.S.A.) on Tenax

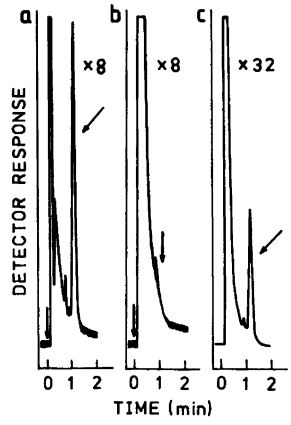

Fig. 1. Gas chromatographic quantitation of ethyl nitrite using electron capture detection. Arrows denote (a) 10 ppb of standard, attenuation ×8, (b) <1 ppb for only smoking or only drinking, attenuation ×8, (c) 37 ppb for Subject A in experiment g (i), attenuation ×32. Operating conditions: sample size, 500 μl; column, glass column (1 m × 1.8 mm I. D.) packed with 6 % poly-m-phenylether, 1 % H_3PO_4 on Tenax (60/80 mesh); column temperature, isothermal at 85 °C; carrier gas (nitrogen) flow rate of 20 ml/min; make-up gas (nitrogen) flow rate of 40 ml/min; detector temperature, 105 °C.

(60/80 mesh), and more conclusively by single ion monitoring at $m/e=60$ during GC/MS of samples and standards.

DISCUSSION

We note that although methyl nitrite has been reported as a component of tobacco smoke[6] ethyl nitrite in breath after only smoking was not detected at concentrations of greater than one ppb. Only in the presence of ethanol does the ethyl nitrite appear. Additional definitive conclusions are not warranted by these few data.

The concentrations of ethyl nitrite are dependent upon individual degree and manner of smoking and drinking as well as upon the solubility of ethyl nitrite in body fluids. Further, for many minutes after ceasing to smoke and drink, ethyl nitrite is still being released from the body. Our sole intent in these experiments was to establish the presence or absence of *in vivo* synthesis and no further experiments are planned.

In a recent review Doll[7] notes that the clearest evidence for the synergistic effect of alcohol/smoking upon the incidence of cancer has been obtained by the International Agency for Research on Cancer in Normandy and Brittany[8] but that the epidemiological evidence tells little about possible mechanisms. Suggested hypotheses were alcohol serving as a carrier for carcinogenic substances in tobacco smoke and alcohol serving as an inducer for the production of enzymes that then activate precarcinogens. Since there is a 90 % correlation between carcinogenicity and mutagenicity of chemicals in the Ames test,[9] this present report of the *in vivo* synthesis of mutagenic ethyl nitrite may explain at least some of that synergism. Finally, we suggest that in attempting to explain at least synergistic effects chemical reactions between system components be considered more frequently.

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