figurations of the 2-butyl carbon atom and phosphorus atom in (−)-*O*-2-butyl *S*-2-(ethylthio)ethyl (−)-ethylphosphonothioate (1a) and (−)-*O*-2-butyl *S*-2-(dimethylammonium)ethyl (−)-ethylphosphonothioate hydrogen oxalate (2a) also are assigned the configurations R_CS_P. The configurations of the remaining chiral isomers may be deduced from their respective optical rotations.

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Rapid Determination of Maleic Hydrazide in Cigarette Smoke Condensate and Particulate Matter

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione), a systemic plant growth regulator, is used extensively as a tobacco sucker inhibitor. A rapid method has been developed to determine quantities of maleic hydrazide (MH) in tobacco smoke condensates. The method involves microcolumn chromatography on alumina, derivatization to form the bis(trimethylsilyl) derivative, and quantitation by gas chromatography. This method has been applied to both cigarette smoke condensate and total particulate matter of cigarette smoke. Various aspects of methodology as well as MH transfer rates from cigarettes into smoke are discussed. Significantly, more than 99% of the MH content of cigarette tobacco was destroyed during smoking.

Maleic hydrazide (MH; 1,2-dihydro-3,6-pyridazinedione), a systemic plant growth regulator, is used by tobacco farmers throughout the world to inhibit growth of suckers on tobacco plants (Tso, 1972). It is usually applied to the upper half or third of the tobacco plant within 24 hr after topping. Subsequently, due to absorption and translocation, MH is found throughout the entire plant. Interest in MH contents of tobacco and tobacco smoke has arisen due to future export restrictions on agricultural chemical residues on tobacco and possible tumorigenic properties of MH in animal tests (Epstein *et al.*, 1967; Epstein and Mantel, 1968).

In a recent paper (Haeberer *et al.*, 1974), a new gas chromatographic method for the analysis of MH residues in tobacco was reported. This method was a marked improvement over earlier optical methods (Wood, 1953; Anglin and Mahon, 1958; Lane *et al.*, 1958; Hoffman, 1961). Although this method (Haeberer *et al.*, 1974) produced excellent results for MH levels in tobacco, its application to cigarette smoke condensate (CSC) or total particulate matter (TPM) was complicated by interfering compounds, which could not be resolved by gas chromatography.

This report details a rapid, quantitative method for determination of maleic hydrazide in both CSC and TPM. The procedure involves microcolumn chromatography of the crude CSC or TPM, derivatization of partially purified MH, and final separation and quantitation by gas chromatography using a flame-ionization detector. Concentrations as low as 0.1 μg of MH in 1 g of CSC have been determined. MH transfer rates from cigarettes to smoke were studied for fortified and original cigarettes.

EXPERIMENTAL SECTION

Reagents. Ethyl acetate was purchased as the analytical reagent grade (Mallinckrodt Chemical Works), dimethylformamide as the spectrophotometric grade (J. T. Baker Chemical Co.), *N,O*-bis(trimethylsilyl)acetamide as the specially purified grade (Pierce Chemical Co.), and 100/120 mesh AG-7 alumina in the fully activated form (Bio-Rad Laboratories). Maleic hydrazide was purchased in the practical grade (Eastman Kodak Co.) and recrystallized twice from distilled water.

Apparatus. A Varian Aerograph gas chromatograph Model 2800, with flame ionization detectors and glass injector liners, was used for the analysis. The separation was performed on 20% OV-11 on Chromosorb W-HP (100–120 mesh). Gas chromatograms were integrated with an Infotronic Model CRS-204 electronic digital integrator.

Cigarettes and Smoke Condensate Preparation. Cigarette smoke condensate and University of Kentucky experimental reference cigarettes (85-mm, nonfilter, type 1R1) (Atkinson, 1970; Benner, 1970) were used for the development of the analytical method and for the determination of the MH transfer rates, respectively. CSC was prepared at the Roswell Park Memorial Institute from commercial 85-mm nonfilter cigarettes. Cigarettes were fortified with 0.1, 0.2, 0.4, 0.8, and 1.6 mg of MH by uniform syringe injection (Lakritz, 1973) of MH solutions. Cigarettes were conditioned at 21° and 60% relative humidity for 62 hr, then smoked on a Mason 24-port smoking machine at a standard rate (one puff/min, 2-sec duration, 35-ml puff volume) to a butt length of 23 mm. The mainstream smoke was drawn through a Cambridge-filter assembly and the smoke particulate matter trapped on a tared filter pad. Immediately after smoking, the pads were weighed and extracted with dimethylformamide (DMF) in a micro Soxhlet apparatus for 4 hr. About 25 ml of DMF was used per g of TPM. After extraction, the concentrations of the DMF solutions were adjusted to 30% TPM based on original TPM weight, by evaporation of DMF on a hot plate (100°), and diluted to volume with DMF.

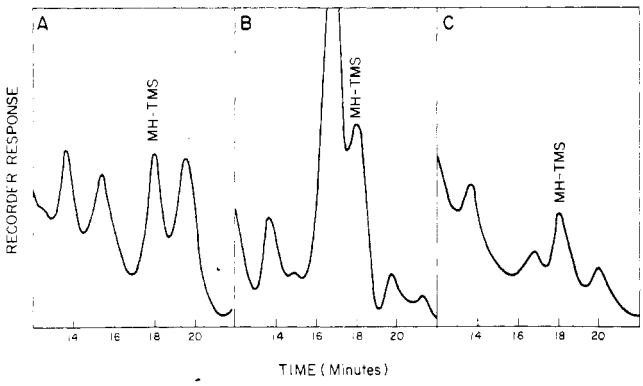

Figure 1. Chromatograms of BSA derivatized samples: (A) 7.5 × 10^{-8} g of MH derived from 5 × 10^{-4} g of pulverized cigarette tobacco; (B) 7.5 × 10^{-9} g of MH derived from 3.75 × 10^{-4} g of cigarette smoke condensate, before cleanup; (C) 7.5 × 10^{-9} g of MH derived from 3.75 × 10^{-4} g of cigarette smoke condensate, after cleanup.

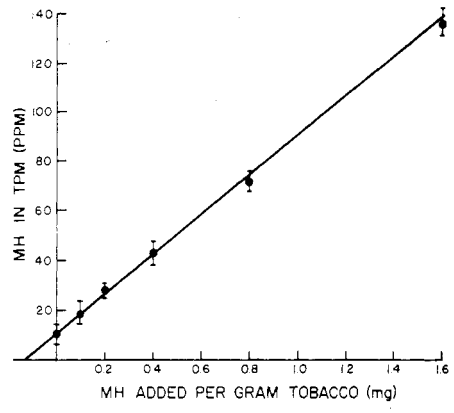

Figure 2. Transfer of maleic hydrazide from cigarettes to the total particulate matter of cigarette smoke.

Column Chromatography and Derivatization. DMF solutions containing 30% CSC or TPM were applied in single 50-μl portions to individual microalumina columns. The micro columns were constructed either from disposable glass transfer pipets or medicine droppers plugged with glass wool and charged with 0.15 g of alumina. The DMF solvent was evaporated from the micro column by drying at 140° for 1 hr. The cooled column was eluted with 5-ml quantities of ethyl acetate and redried at 140° for 30 min. The alumina was then transferred into a 0.3-ml reaction vial equipped with a Teflon-lined cover, capable of being sealed pressure tight. *N,O*-Bis(trimethylsilyl)-acetamide (200 μl) was added and the vessel tightly sealed, shaken vigorously, and placed on a hotplate, set at 100°, for 30 min. The cooled supernatant liquid was subsequently subjected to gas chromatographic analysis.

Gas Chromatography. The stainless steel column, 2 mm × 3 m, filled with 20% OV-11 on Chromosorb W-HP (100–120 mesh), was conditioned as described previously (Haeberer *et al.*, 1974). The helium (zero gas) flow rate was 30 ml/min. The column, injector, and detector temperatures were 160, 220, and 240°, respectively. At these conditions, the retention time for the bis(trimethylsilyl) derivative of MH (MH-TMS) was about 18 min (Kovats retention index 1462). To prevent possible degradation of the MH-TMS derivative, glass liners were installed in the injector ports. Concentrations as low as 0.1 μg of MH/g of TPM or CSC produced sufficient signal for quantitation.

RESULTS AND DISCUSSION

Attempted application of our original method for MH in tobacco (silylation of MH and subsequent gas chromatography) to the analysis of MH in crude CSC was not immediately successful. Direct silylation of CSC or TPM with *N,O*-bis(trimethylsilyl)acetamide (BSA) yielded gas chromatograms similar to those shown in Figure 1B. The analysis was complicated by interfering CSC constituents, not present in tobacco extracts. Removal of interfering substances was accomplished by tlc on alumina with ethyl acetate. The alumina from the origin, containing the MH as well as some other CSC compounds, was scraped off the tlc plate, placed into a reaction vial, and treated with silylating agent (BSA). Gas chromatography of the MH-TMS on OV-11 showed that MH was successfully recovered from the alumina and derivatized quantitatively, in comparison with MH standards that were directly derivatized.

Utilizing this strong affinity of alumina for MH, column chromatography was explored. Small columns of alumina (0.15 g) were prepared from disposable glass transfer pipets or medicine droppers. Known quantities of MH in DMF were applied; the columns were heated to remove the DMF and eluted with various solvents. Only ethyl alcohol and more polar solvents removed detectable amounts of the applied MH. This column chromatographic procedure was then applied to CSC with the objective of removing the interfering condensate constituents, while still retaining the MH on the alumina. Purification of MH was most successful with ethyl acetate, acetone, and *n*-propyl alcohol. Silylation of the MH–alumina mixtures and subsequent gas chromatographic analysis of the MH-TMS derivative yielded chromatograms with good resolution (Figure 1C). Thus, the final procedure for MH analysis consisted of sample adsorption on alumina, elution of interferences with ethyl acetate, extraction and derivatization with the BSA reagent, and gas chromatography on OV-11. For samples of CSC tested, MH concentrations ranged from 11 to 20 ppm.

A recovery study was also conducted of known amounts of MH in both pure DMF and in DMF solutions containing 30% CSC. These solutions were applied directly to the alumina in the columns and treated as described above. Recoveries proved to be quantitative when compared to MH standards that were directly derivatized. During the preparation of this manuscript, Liu and Hoffmann (1973) published a paper on the determination of MH in smoke condensates. This method involves a variety of techniques, including ion exchange chromatography, reaction with 4-chlorobenzyl chloride, absorption chromatography, and gas chromatography using a ^{63}Ni-electron capture detector. This procedure appears to be more complicated and more time consuming as compared to the currently reported method.

After development of this improved method it was decided to examine the transfer of MH from cigarette tobacco to smoke. Factors affecting amounts of MH transferred, such as draw resistance, burning rate, and temperature, were not investigated. Only the quantity of MH transferred from the cigarettes to smoke was determined. It was necessary to know the exact amount of MH in the unfortified cigarettes. However, that information was not available for the cigarettes used to prepare the CSC employed in the development of this method. Therefore, experimental reference cigarettes, type 1R1, manufactured at the University of Kentucky, were employed to measure the transfer of MH to smoke. It was also decided to determine the MH content of TPM of cigarette smoke rather than that of whole CSC; a representative sample of TPM can be obtained from as few as four cigarettes. Total particulate matter represents only a portion of CSC since the latter is trapped at −78° while TPM is removed by filtration at ambient temperature.

The cigarettes were fortified with standard quantities of MH, conditioned, and smoked. The results of the analyses on the DMF extracts of filters, used to trap the TPM, are

presented in Figure 2. Extrapolation of the data indicated that unfortified 1R1 cigarette tobacco contained about 130 ppm of MH, which is in fair agreement with the 103 ppm reported earlier (Haeberer *et al.*, 1974). About 55 μg of MH per unfortified 1R1 cigarette, or about 0.5%, was transferred to mainstream smoke. Apparently, about 99% of the MH in cigarette tobacco is pyrolytically degraded into other products, assuming that sidestream smoke does not contain a significantly different MH concentration.

Finally, it was of interest to determine whether or not any MH distills ahead of the burning cone of a cigarette. If this were the case, then considerable quantities of MH would be found in the butts. Cigarette butts from unfortified and fortified cigarettes (0.8 or 1.6 mg added MH/g of tobacco) were analyzed for MH content. Butts with 1.6 mg of MH added contained 0.4 mg of MH. A 23-mm butt represents about 27% of an 85-mm cigarette, and should contain about 27% of the applied MH. The analysis showed that the butt contained 25% of the MH. This indicated that MH does not distill ahead of the burning zone to be condensed and concentrated in the butt. Since distillation can now be ruled out, the disperse phase or aerosol particles must be responsible for transport of MH into smoke.

The behavior of MH under pyrolytic conditions could be anticipated from its stability during melting point determinations. It was observed that MH does not melt but degrades at 296°. This substantiates our findings of pyrolytic decomposition of MH in the burning cigarette.

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Chloroanisoles as Off-Flavor Components in Eggs and Broilers

Musty eggs and broilers were analyzed for the presence of chloroanisoles and chlorophenols. The taint was caused by tri- and tetrachloroanisole. The contamination of the poultry products was brought about either by the use of woodshavings or by the use of contaminated feed.

In 1972 and 1973 a distinct musty taint was often observed in eggs and broilers from poultry farms in different parts of The Netherlands.

In earlier investigations (Engel *et al.*, 1966; Curtis *et al.*, 1972) it was shown that a musty taint in eggs and broilers was caused by 2,3,4,6-tetrachloroanisole and pentachloroanisole. These compounds were also found to be present in litter and woodshavings from the relevant poultry houses where they were picked up by the chickens. It was assumed, therefore, that the odor was thus transferred to the eggs and the carcasses.

It has been proved by Curtis *et al.* (1972) that the presence of chloroanisoles is caused by the ability of the microorganisms in the litter to form 2,3,4,6-tetrachloroanisole and pentachloroanisole from the corresponding chlorophenols. Furthermore, Cserjesi and Johnson (1972) found that *Trichoderma* sp. were able to convert pentachlorophenol partially into pentachloroanisole.

Technical grade pentachlorophenol, which is used as a wood preservative and contains up to 13% impurities, mainly isomeric tetrachlorophenols (Melnikov, 1971), may act as a precursor for the chloroanisoles. In addition, Ide *et al.* (1972) found that pentachlorophenol itself was decomposed by microorganisms into mono-, di-, tri-, and tetrachlorophenols.

Since chlorophenols may act as precursors for the corresponding chloroanisoles, both the presence of chloroanisoles and chlorophenols was determined in the products in question. The results of the investigation are described here.

Woodshavings and tainted products from poultry houses were analyzed. Since tainted products were also produced in poultry houses where no woodshavings were used, feed samples and in a few instances some of the raw materials used in the feed were examined as well.

MATERIALS AND METHODS

The methods applied to concentrate the chloroanisoles and chlorophenols were specific for each type of product. Eggs having a musty taint were selected from suspected lots. As the taint was located in the egg yolks, only this fraction was subjected to analysis. The yolk samples were saponified in an alcoholic KOH solution and subsequently extracted with ether. The extract was washed with water until all alkali was removed.

Tissue samples of tainted broilers, mainly consisting of depository fat and skin, as well as feed samples, were steam distilled and extracted with pentane–ether in an apparatus described by Likens and Nickerson (1964). The woodshavings were extracted in a Soxhlet apparatus. All extracts, which were dried over sodium sulfate and concentrated by distilling off the solvents, were used for mass spectrometric analysis.

Samples of animal grease, used for the preparation of feed, were dried over sodium sulfate and subjected for 2.5 hr to the high vacuum sublimation procedure of Lea and Swoboda (1962) at 80°. The volatile material, which was condensed on a cold finger, was washed off with ether and used for analysis.

Fractions of the concentrates were introduced with a cold probe into the ionization chamber of a double focus-