0-, 10-, 30-, and 100-ppt level and submitted to a panel of 10 tasters in a blind fashion. The tasters were asked to compare the fortified samples to reference samples of the original wines. All persons recognized a cork taint in wines spiked at the 100-ppt level. An off-flavor was still recognized at the 30- and 10-ppt level by nine and five persons, respectively. These preliminary results indicate the extreme potency of 2,4,6-TCA and show that concentrations as low as 10 ppt can be perceived organoleptically and thus diminish the quality of wine. The actual amounts of 2,4,6-TCA recognizable by the human nose apparently are as low as a few picograms (10^{-12} g).

Additional chlorinated compounds were detected in treated cork by using GC–MS. They included dichloroanisole, mono-, di-, and trichlorophenol, mono- and dichloromethoxyphenol (guaiacols), di- and trichlorodimethoxybenzene (veratroles), and chloronaphthol. The significance of all these compounds for the cork taint in wine remains to be investigated.

CONCLUSIONS

2,4,6-TCA was found as a major component responsible for the musty cork taint occasionally found in cork-bottled wines. Cork taint in wine was caused from concentrations of 2,4,6-TCA in the ppt range and proved the extreme potency of this compound. Although 2,4,6-TCA could be perceived with ease by the human nose at these extremely low concentrations, the detection and identification required sophisticated and extremely sensitive analytical techniques such as high-resolution gas chromatography and mass spectrometry. The presence of 2,4,6-TCA at these small levels in wine very likely poses no toxicological risk; nevertheless, it completely destroys the quality of this product.

The origin of 2,4,6-TCA in wine is not yet fully known. Because larger quantities of this and related chlorinated compounds (chlorinated anisols, phenols, guaiacols, and veratroles) were detected in cork used for bottling of wine, the involvement of this material is strongly suggested. The occurrence of 2,4,6-TCA and these related compounds could possibly arise from the chlorination of lignin-related compounds during chlorine bleaching used in the processing of cork and later extraction of these compounds into the wine. If this proves to be true, replacement of chlorine treatment in the processing of cork should remedy the cork-taint problem.

In further experiments, the occurrence of 2,4,6-TCA and the other extraneous compounds should be documented. Simplified analytical methods (e.g., electron-capture detection of 2,4,6-TCA) could prove to be suitable for this purpose. Such analyses could supplement but hardly replace organoleptic tests. We believe that finding 2,4,6-TCA as a major cause for cork taint in wine is an important contribution in continuing efforts to maintain the quality and purity of this product.

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2-Acetyl-5-chloropyrrole in the Volatile Flavor Constituents of Cocoa Butter

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The presence of 2-acetyl-5-chloropyrrole in the volatile flavor constituents of cocoa butter was confirmed through synthesis of the authentic compound. This compound was synthesized by chlorination of 2-acetylpyrrole. The structure of the synthesized compound was established by infrared, nuclear magnetic resonance, and mass spectrometry. The identification of this compound in the volatile flavor constituents of cocoa butter was confirmed by comparing the mass spectrum and gas chromatographic retention time with those of the authentic sample. 2-Acetyl-5-chloropyrrole is the first chlorinated heterocyclic compound identified in the volatile flavor of foods.

The isolation and identification of the volatile flavor constituents from cocoa butter have been recently described (Ho et al., 1981). The present paper reports confirmation of the presence of 2-acetyl-5-chloropyrrole through synthesis of the authentic compound.

The presence of halogenated aliphatic and aromatic hydrocarbons has been reported in the volatiles of various foods, such as baked potatoes (Ho and Coleman, 1981), canned beef stew (Chang and Peterson, 1977), vinegar (Kahn et al., 1972), and cheeses (Dumont et al., 1974a,b). These halogenated hydrocarbons may be undesirable to human health (Fishbein, 1979a,b). Heterocyclic compounds are widely distributed in food aromas. However, no halogenated heterocyclic compounds have been identified.

The synthesis of 2-acetyl-4-chloropyrrole and 2-acetyl-5-chloropyrrole was undertaken in order to determine the exact structure of a monochloroine-substituted acetylpyrrole in the volatile flavor constituents of cocoa butter.

EXPERIMENTAL SECTION

Synthesis of 2-Acetyl-5-chloropyrrole and 2-Acetyl-4-chloropyrrole. 2-Acetylpyrrole was purchased

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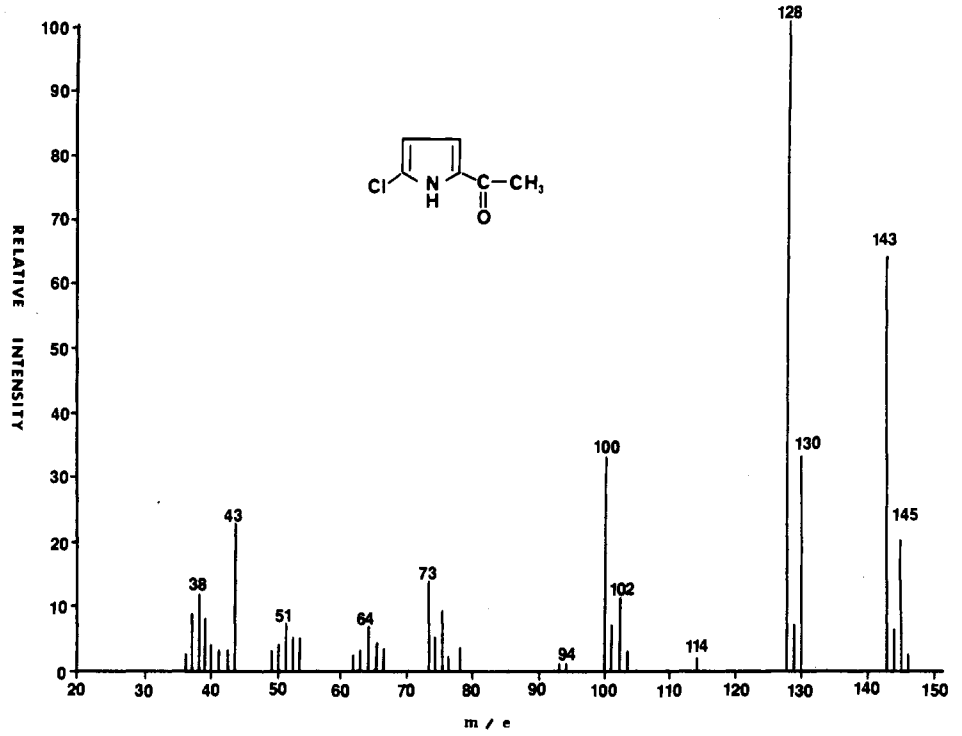

Figure 1. Mass spectrum of 2-acetyl-5-chloropyrrole.

from Tridom Chemicals, Inc., New York.

To 0.49 g (4.5 mmol) of 2-acetylpyrrole in 20 mL of diethyl ether at 0–5 °C, 0.67 g (5 mmol) of sulfuryl chloride in 10 mL of diethyl ether was added dropwise. After addition, the mixture was stirred for 2 h at 0–5 °C. To the reaction mixture, at room temperature, 10 mL of a 10% sodium carbonate solution was added. The ethereal layer was separated and washed with water. The solution was dried over anhydrous sodium sulfate and the solvent removed with the use of a rotary evaporator. A colorless crystalline material was obtained by steam distillation.

Separation and Purification of 2-Acetyl-5-chloropyrrole and 2-Acetyl-4-chloropyrrole. 2-Acetyl-5-chloropyrrole and 2-acetyl-4-chloropyrrole were separated on a Beckman GC-55 gas chromatograph by using a 12 ft × $^1/_4$ in. preparative column of 10% OV-17 on 80–100-mesh Chromosorb W. The following conditions were employed: initial time, 1 min; temperature program, 80–220 °C at 7.5 °C/min; final time, 2 min; flow rate (He), 40 mL/min.

The retention times of the separated samples were 11.5 min for 2-acetyl-5-chloropyrrole and 14.2 min for 2-acetyl-4-chloropyrrole. The ratio of 2-acetyl-5-chloropyrrole to 2-acetyl-4-chloropyrrole was 1.6:1.

Identification of 2-Acetyl-5-chloropyrrole and 2-Acetyl-4-chloropyrrole. The mass spectra were obtained on a Du Pont 21-490 mass spectrometer. Nuclear magnetic resonance spectra were obtained on a Varian CST-20 pulse nuclear magnetic resonance spectrometer, and infrared spectra were obtained on a Beckman Acculab 4 IR spectrometer by using ultramicro sodium chloride cells of 0.1-mm light path.

RESULTS AND DISCUSSION

The mixture of 2-acetyl-5-chloropyrrole and 2-acetyl-4-chloropyrrole was synthesized by the reaction of 2-acetylpyrrole with sulfuryl chloride. It was established that only the 4 and 5 positions of the pyrrole ring would be chlorinated if there was an electron-withdrawing group, such as an acetyl group, present at the 2 position of the pyrrole ring (Hodge and Rickards, 1965; Anderson and Lee, 1965).

The 2-acetyl-5-chloropyrrole and 2-acetyl-4-chloropyrrole, purified and separated by GC, were analyzed by IR, NMR, and mass spectrometry. The orientation of chlorine atom in the pyrrole ring was established by NMR spectrometry. In pyrrole itself, H-2 and H-5 resonate at δ 6.60 and H-3 and H-4 at δ 6.13 (Jackman, 1959). The introduction of a chlorine substituent into the pyrrole ring was found to cause only minor shifts in the resonance frequencies of the remaining hydrogens (Hodge and Rickards, 1965). However, the presence of a diamagnetically anisotropic carbonyl function in the 2 position would deshield strongly the adjacent H-3, while only weakly deshielding H-5 and scarcely affecting H-4 (Hodge and Rickards, 1965). Thus, the structure of 2-acetyl-4-chloropyrrole is clearly recognized by its NMR spectrum in which both ring protons resonated at the low-field region of δ 6.8 and 6.95. The two ring protons of 2-acetyl-5-chloropyrrole resonated at δ 6.15 and 6.8 in its NMR spectrum.

The chlorinated acetylpyrrole isolated from the volatiles of cocoa butter had the same retention time in the gas chromatogram as the synthesized 2-acetyl-5-chloropyrrole. The mass spectrum of 2-acetyl-5-chloropyrrole (Figure 1) also matched well with that of the isolated compound. The ratio of the m/e 145 ion to the m/e 143 ion was calculated to be 32.8% which clearly indicated the presence of a chlorine atom. The base peak at m/e 128 was due to the loss of CH_3· from the acetyl group of the molecular ion. The fragment ion at m/e 100 (loss of CH_3CO from the molecular ion) and m/e 43 (acetyl ion) also indicated the presence of an acetyl group. It is therefore concluded that the chlorinated pyrrole compound isolated from the volatiles of cocoa butter was 2-acetyl-5-chloropyrrole. The infrared spectrum of 2-acetyl-5-chloropyrrole showed two strong peaks at 3438 and 3215 cm^{-1} which indicated the N–H stretching in the pyrrole ring. The strong absorption at 1644 cm^{-1} indicated the presence of a carbonyl group.

2-Acetyl-5-chloropyrrole had a strong, almond aroma. Formation of this compound in cocoa butter awaits a detailed mechanistic study.

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A Rapid Liquid–Liquid Extraction Cleanup Method for the Determination of Volatile *N*-Nitrosamines in Cooked-Out Bacon Fat

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A rapid liquid–liquid extraction method is described for the determination of volatile nitrosamines in cooked-out bacon fat. The method consists of partitioning of the nitrosamines between *n*-hexane and an acidic aqueous–methanol mixture containing small amounts of sulfamic acid. An aliquot of the aqueous phase is then extracted with dichloromethane, the dichloromethane extract concentrated, and an aliquot of the concentrated extract analyzed by a GLC–thermal energy analyzer. The average percentage recoveries of *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosopiperidine, *N*-nitrosopyrrolidine, and *N*-nitrosomorpholine when added to cooked-out bacon fat or lard at levels ranging between 5 and 20 ppb were 78.8, 77.8, 89.4, 100.3, and 97.4, respectively. The method has an overall detection limit of 1 ppb for each of the above five nitrosamines. The average levels (uncorrected) of *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine detected in the 11 samples of cooked-out bacon fat were found to be 4.8 and 21.1 ppb, respectively.

Studies during the past 10 years have shown that fried bacon is one of the food products that consistently contains traces of volatile *N*-nitrosamines (simply called nitrosamines), mainly *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosodimethylamine (NDMA). These studies (Sen, 1980) have also indicated that the concentration of volatile nitrosamines in the cooked-out bacon fat is approximately twice that present in the cooked bacon. Since both NDMA and NPYR are potent carcinogens, it would be desirable to have a rapid and sensitive analytical method for monitoring their presence in these and other food items. Although a variety of methods are presently available (Preussmann et al., 1978) for this purpose, many of them are too lengthy and some need expensive instrumentation such as high-resolution mass spectrometry. Of the available methods, the combined gas–liquid chromatographic–thermal energy analyzer (GLC–TEA) technique seems to be best suited for routine monitoring. In the GLC mode, the TEA detector is highly sensitive and specific for nitrosamines (Fine et al., 1975b) and has the added advantage that it can also be operated in the high-pressure liquid chromatographic (HPLC) mode which is sometimes useful for confirmation of the GLC data. The detector is expensive but not beyond the reach of most modern laboratories.

Because of the extremely high specificity of the TEA detector, it is not always necessary to carry out extensive sample cleanup prior to the end determination by GLC–TEA. For example, two rapid Celite column cleanup methods for the determination of volatile nitrosamines in fried bacon (Fiddler and Pensabene, 1980) and beer (Hotchkiss et al., 1981) have already been reported. The above-mentioned method for fried bacon, however, was reported to be unsatisfactory for the analysis of cooked-out bacon fat. It should also be noted that the mineral oil distillation method, which is used by many laboratories (Fine et al., 1975a; Havery et al., 1978) for the analysis of a variety of foods for their nitrosamine contents, has been reported to produce inconsistent and low results (as low as 17% recovery) when applied to cooked-out bacon fat (Owens and Kinast, 1980). We report here a rapid sample workup procedure suitable for the analysis of this product.

EXPERIMENTAL SECTION

Samples. The samples were purchased locally in the Ottawa area. Bacon slices were fried in a Teflon-coated electric frypan (340 °F setting) as described previously (Sen et al., 1979). After the slices were fried, the

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