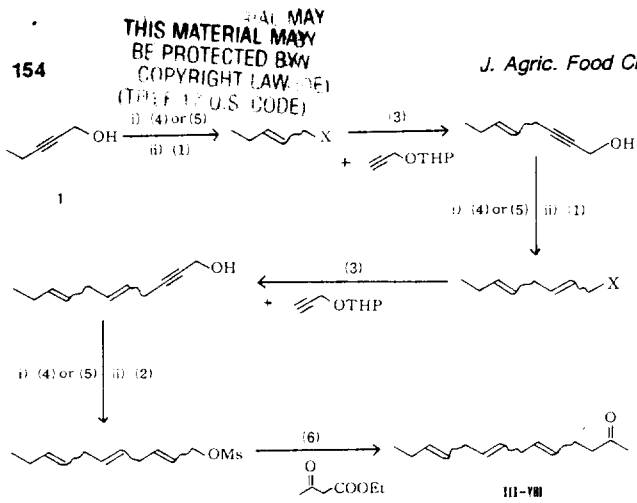

Figure 3. General synthetic routes to the geometrical isomers of 5,8,11-tetradecatrien-2-one (III–VIII).

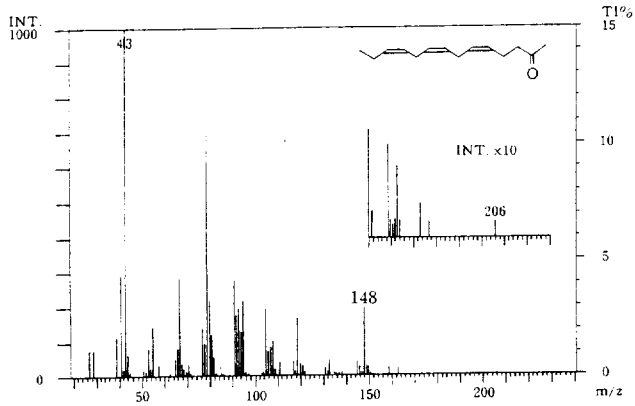

Figure 4. Mass spectrum of synthetic (5*Z*,8*Z*,11*Z*)-tetradecatrien-2-one.

termediate of these values, 30 ppm, between *E* and *Z*.

From these results, it became possible to determine the stereostructures of 5,8,11-tetradecatrien-2-one isomers from their ^1H and ^{13}C NMR spectra. The NMR data for the natural products of C_{14} ketones were described in the preceding report, and they coincided with those of I and II.

Sensory Description. The described aroma characteristics of the eight isomers are summarized in Table I. It is interesting that two natural types of ketones (I and II) had a seafood aroma reminiscent of cooked small shrimp and shellfish. As compounds III and V also had an odor like seafood products, the presence of the C-11 *Z* double bond seems to have been necessary to show the common aroma characteristics of seafood products. Considering that the other isomers showed little difference in the odor from those of natural seafood materials and that the unconjugated *Z* double bonds separated by methylene is a typical partial structure of natural unsaturated fatty acid, a biosynthetic study of the C_{14} ketones would be advisable not only for the flavor formation but also for the lipid metabolism. We are continuing our research in this direction.

Registry No. 1, 6261-22-9; 1 (bromo derivative), 16400-32-1; 2, 6089-04-9; 3, 35378-76-8; 3 (THP derivative), 117606-23-2; 4, 1558-79-8; 5, 42541-87-7; 6, 101339-85-9; 7, 67548-44-1; 8a, 105894-11-9; 8b, 117606-24-3; 9, 101339-95-1; 10a, 101339-96-2; 10b, 117606-25-4; I, 85416-33-7; II, 101339-97-3; III, 105657-89-4; IV, 117606-22-1; V, 105657-91-8; VI, 105657-92-9; VII, 105657-90-7; VIII, 85421-52-9; (2*Z*,5*E*,8*Z*)-$CH_3(CH_2CH{=}CH)_2CH_2OMS$, 117606-26-5; (2*Z*,5*Z*,8*E*)-$CH_3(CH_2CH{=}CH)_3CH_2OMS$, 117606-27-6; (2*E*,5*E*,8*Z*)-$CH_3(CH_2CH{=}CH)_3CH_2OMS$, 117606-28-7; (2*E*,5*Z*,8*E*)-$CH_3(CH_2CH{=}CH)_3CH_2OMS$, 117606-29-8; (2*Z*,5*E*,8*E*)-$CH_3(CH_2CH{=}CH)_3CH_2OMS$, 117606-30-1; (2*E*,5*E*,8*E*)-$CH_3(CH_2CH{=}CH)_3CH_2OMS$, 117606-31-2; CH_3COCH_2COOEt, 141-97-9; (5*E*,8*Z*)-$CH_3(CH_2CH{=}CH)_2CH_2C{\equiv}CCH_2OH$, 117606-32-3; (5*Z*,8*E*)-$CH_3(CH_2CH{=}CH)_2CH_2C{\equiv}CCH_2OH$, 117606-33-4; (5*E*,8*E*)-$CH_3(CH_2CH{=}CH)_2CH_2C{\equiv}CCH_2OH$, 101339-91-7; (*Z*)-$CH_3CH_2CH{=}CHCH_2C{\equiv}CCH_2OH$, 117606-34-5; (*E*)-$CH_3CH_2CH{=}CHCH_2C{\equiv}CCH_iOH$, 101339-87-1.

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Analyses of Steam Distillates and Aqueous Extracts of Smokeless Tobacco

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The steam distillates or aqueous extracts of nine brands of commercial snuff were analyzed in an effort to determine those constituents present as additives. These extracts were then partitioned with methylene chloride and profiled on capillary GC. These profiles were compared with those obtained with extracts from 1S3 tobacco, the University of Kentucky's reference research tobacco for moist snuff. Those compounds unique to the commercial brands or present in exceptional quantities relative to 1S3 tobacco were identified by GC–MS. Quantitative analyses were performed by selective ion monitoring/mass spectrometry. Methyl salicylate, ethyl salicylate, benzyl benzoate, phenylethanol, geraniol, citronellol, acetovanillone, syringaldehyde, and acetylpyridine were among the compounds identified and quantitated in commercial moist snuff.

Several epidemiological studies have reported an association between the use of smokeless tobacco and oral cancer (Ahlbom, 1937; Rosenfeld and Callaway, 1963; Winn et al., 1981). The International Agency for Cancer Re- snuff were 68%, 79%, and 78%, respectively. Under the experimental conditions employed for the aqueous extractions, recoveries of these esters were only 47%, 14%, and 11% respectively.

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search has concluded that there is "sufficient evidence to indicate a causal relationship between the use of snuff and oral cancer" (International Agency for Research on Cancer, 1985).

The major constituents of snuff, including additives, would be available in highest concentration at those sites where preneoplastic and neoplastic lesions associated with smokeless tobacco usage appear. Several carcinogens have previously been identified as constituents of commercial snuff tobaccos (Hoffmann et al., 1986, 1987). The present study was performed to identify additives, as well as other constituents unique to commercial snuff, which may have the potential to influence the genotoxic effects of known tobacco carcinogens.

The manufacturing of the commercial snuff tobacco blends requires extensive curing and fermentation processes and frequently includes additives. Thus, the constituents of snuff tobacco can vary widely from those present in either burley, oriental, or flue-cured cigarette tobaccos. The University of Kentucky's research reference tobacco for moist snuff, 1S3, served as the basis for comparison with nine commercial brands of moist snuff. In these analyses we compare the constituents of steam distillates obtained from acidified tobaccos as well as from an aqueous extract of the tobacco.

EXPERIMENTAL SECTION

Apparatus. Gas chromatographic profiles of the steam distillates or of tobacco extracts were obtained with a Hewlett-Packard Model 5790A gas chromatograph equipped with a split–splitless injector, flame ionization detector, and a Model 3390A calculating integrator, on a 50 m × 0.25 mm (i.d.) OV-101 fused silica capillary column. The conditions employed for these analyses were as follows: injection port, 200 °C; detector, 250 °C; gradient from 60 to 230 °C at 2 °C/min with a helium flow of 1 mL/min and a split ratio of 20:1. Mass spectra were obtained on a Hewlett-Packard Model 5988A dual-source GC-MS with a HP 1000 computer system. The gas chromatographic conditions were as described above, with the capillary direct interface being heated to 280 °C. The ion source of the mass spectrometer was heated to 200 °C and operated at 70 eV. For the accumulation of mass spectra, scans were made between 45 and 450 amu.

Procedures. Commercial snuff products were purchased on the open market in Westchester County, NY, during 1987. The 1S3 reference moist snuff was obtained from the University of Kentucky. This reference material is composed of 25.7% dark-fired tobacco, 7.8% air-cured tobacco, 3.7% burley stems, 0.5% sodium carbonate, 7.4% NaCl, and 54.8% H_2O. It was packaged in plastic containers containing 1.2 oz of snuff. The steam distillates from snuff tobaccos were prepared as described previously (LaVoie et al., 1985).

Aqueous extracts of tobacco were prepared by stirring 5.0 g of tobacco in 250 mL of water for 12 h at room temperature. The mixture was then filtered and the aqueous solution extracted with three equal volumes of methylene chloride. The combined methylene chloride extracts were concentrated as above to 0.1 mL and analyzed by GC and GC–MS.

The quantitative determination of specific constituents was done by selective ion monitoring (SIM)/mass spectrometry. Peak areas were compared with standard curves generated from mixtures of reference standards at varying concentrations. The quantitative analyses performed in triplicate on a steam distillate obtained from two brands of tobacco resulted in the detection of 11.3 ± 2.8 mg of ethyl salicylate in one of the brands that contained the highest levels of this compound and the detection of 2.7 ± 0.9 μg of methyl salicylate in a brand that contained relatively low levels of this flavorant.

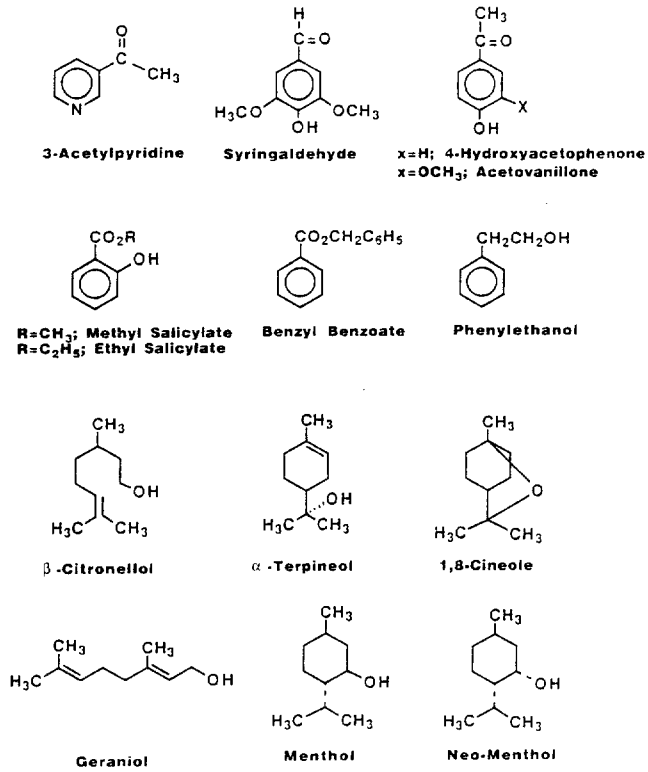

Figure 1. Structures of constituents identified in commercial U.S. moist snuff brands.

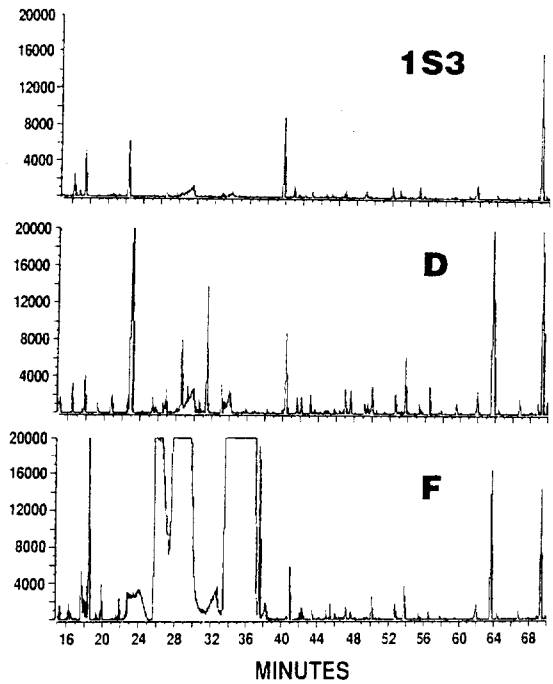

Figure 2. GC–MS profile of steam distillate of 1S3 snuff tobacco and the commercial tobacco brands D and F.

Subfractions of concentrates of the steam distillates and of the aqueous extracts of tobacco were also prepared and analyzed. Each concentrate was diluted to 5.0 mL with methylene chloride and extracted with three equal volumes of 5% aqueous NaOH to remove phenols and carboxylic acids. The methylene chloride layer of this extract contained basic and neutral components. The acidic fraction was obtained by acidification of the aqueous layer with 1.0

Table I. Qualitative Identification of Certain Prominent Constituents of Commercial Snuff Brands

compound	ret time, min	commercial U.S. moist snuff brands								
		A	B	C	D	E	F	G	H	I
benzyl alcohol	17.8							+		
phenol	18.2								+	
cineole	18.5	+				+	+			+
α-terpineol	20.5									+
acetylpyridine[a]	21.8			+	+		+	+	+	+
phenylethanol	22.7	+	+		+		+	+	+	+
menthol	26.0	+				+	+			+
neomenthol	26.9	+				+	+			+
methyl salicylate[b]	28.8	+	+	+		+	+	+	+	+
ethyl salicylate[b]	33.0	+				+	+			+
β-citronellol[a]	35.9				+		+	+		+
geraniol[a]	37.9						+			
acetovanillone	46.7			+	+		+	+		+
4-hydroxyacetophenone	47.8				+		+			
syringaldehyde	56.9						+			
benzyl benzoate[c]	63.6				+		+	+		+
rel moisture, %		31	51	50	52	44	51	50	20	29

[a] Aqueous extract. [b] Salicylates are associated with "wintergreen flavor". [c] Steam distillate.

Table II. Analytical Data on Volatile Constituents of Selected Snuff Products

compound	ions monitored	snuff, μg/g dry tobacco		brands analyzed[b]
		1S3	commercial[a]	
3-acetylpyridine	106, 121	4.2	2.1–18	C, D, F, G, I
phenylethanol[c]	91, 122	32.4	34–210	D, F, G, I
methyl salicylate[c]	92, 120	4.2	62–24 000	C, F, G, I
ethyl salicylate[c]	120, 166	2.7	16.0–14 000	F, G, I
geraniol	69, 93	nd[d]	0.6	F
citronellol	69, 95	nd[d]	1.4–20	F, G, I
acetovanillone	151, 166	0.9	1.3–7.3	C, D, F, G, I
syringaldehyde	181, 182	0.9	49	F
benzyl benzoate[c]	105, 212	nd[d]	30–110	D, F, G, I

[a] The commercial U.S. snuff brands C, D, F, G, and I as listed in Table I were analyzed by SIM for these specific compounds. The levels reported are derived from data obtained from aqueous extracts of tobacco unless otherwise noted. The brand containing the highest concentration of a specific compound was among those selected for these analyses. Among the five commercial snuff tobaccos selected, those that did not contain detectable levels of specific compounds or had relatively minor quantities as compared to the other brands were excluded in the determination of the levels present. [b] These are the select commercial snuff brands as indicated in Table I from which the quantitative range in the levels of individual snuff constituents was developed. [c] The ranges of quantitative values are from the steam distillates of various tobacco blends. [d] nd = not detected (<0.2 μg/g).

N HCl and extraction with three equal volumes of methylene chloride. These methylene chloride extracts were combined, concentrated as previously outlined, dried (Na_2SO_4), and analyzed by capillary GC–FID and GC–MS.

RESULTS AND DISCUSSION

Our study suggests that the use of additives in commercial smokeless tobacco products is responsible for the presence of constituents that are unique or present in exceptionally high quantities relative to the moist snuff reference sample, 1S3. Results of the GC–FID and GC–MS analyses were initially compared for the methylene chloride soluble portion of the steam distillates and aqueous extracts of 1S3 reference snuff tobacco and various commercial snuff tobaccos. The GC profiles revealed the presence of several compounds as either unique to specific commercial snuff tobacco brands or as exceptional in concentration relative to 1S3 tobacco (Figures 1 and 2). These compounds were identified on the basis of the spectra obtained from GC–MS analyses of the tobacco mixtures and by comparison of retention times with those of reference standards.

Qualitative results are summarized in Table I. Using selective ion monitoring, we quantitated the levels of various components in those brands in which their presence was particularly apparent. The tobacco brands that were quantitatively compared to 1S3 tobacco included tobaccos C, D, F, G, and I. The specific ions monitored, the quantitative data, and the brands analyzed are listed in Table II.

Several of the constituents identified as additives or in higher concentration relative to 1S3 tobacco were aromatic compounds. Aryl ketones, such as 3-methoxy-4-hydroxyacetophenone (acetovanillone), 4-hydroxyacetophenone, and 3-acetylpyridine, were identified in certain snuff brands. The levels of individual aromatic ketones ranged from 0.9 to 18.1 μg/g (dry weight) of commercial snuff. Aromatic esters, such as methyl salicylate (wintergreen), ethyl salicylate, 4-hydroxymethyl benzoate, and benzyl benzoate, were also detected in several of the commercial snuff brands. In 1S3 tobacco, methyl salicylate and ethyl salicylate were detected at levels of 2.7 and 4.2 μg/g (dry weight). In contrast, methyl salicylate and ethyl salicylate were present in the commercial tobacco blends at levels as high as 24 000 and 14 000 μg/g (dry weight), respectively. Benzyl benzoate, which was detected only in commercial snuff brands, was present at levels of 30–110 μg/g of tobacco. The extent to which these compounds are present in certain commercial snuff tobaccos as compared to other brands, or 1S3 tobacco, indicates that they are either added directly or as constituents of an extract used in the formulation of these particular snuff brands.

Levels of benzyl benzoate, methyl salicylate, and ethyl salicylate were lower in the aqueous extract than in the steam distillate of a given snuff tobacco. This finding is consistent with recovery studies performed with benzyl benzoate, methyl salicylate, and ethyl salicylate. The recoveries of these esters from steam distillates of 1S3 moist

Other flavor-related components detected in snuff tobacco at exceptionally high concentrations included geraniol, benzyl alcohol, 1,8-cineole, phenylethanol, and syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde). In one of the commercial brands, geraniol was quantified at 0.6 μg/g of tobacco (dry weight) and syringaldehyde at 49 μg/g of tobacco (dry weight). We also detected menthol, isomenthol, and menthone in some of the commercial snuff brands.

Several of the compounds identified in this study (phenol, methyl salicylate, benzyl benzoate), which appear to be additives to commercial snuff tobaccos, are known irritants. These constituents in commercial snuff tobacco can potentially influence the genotoxic activity of the identified tobacco carcinogens, *N'*-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and benzo[*a*]pyrene (Hoffmann et al., 1986, 1987). This possibility is currently being evaluated.

ACKNOWLEDGMENT

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Registry No. Benzyl alcohol, 100-51-6; phenol, 108-95-2; cineole, 470-82-6; α-terpineol, 98-55-5; acetylpyridine, 350-03-8; phenylethanol, 60-12-8; menthol, 89-78-1; neomenthol, 491-01-0; methyl salicylate, 119-36-8; ethyl salicylate, 118-61-6; β-citronellol, 106-22-9; geraniol, 106-24-1; acetovanillone, 498-02-2; 4-hydroxyacetophenone, 99-93-4; syringaldehyde, 134-96-3; benzyl benzoate, 120-51-4.

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Changes in Composition of Volatile Components in Aseptically Packaged Orange Juice during Storage

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A procedure involving low-pressure distillation and capillary gas chromatography of the distillate was used to monitor 29 volatile components of aseptically packaged orange juice during 8 months of storage at 21 and 26 °C. A gradual decrease in several flavor components, 1-penten-3-one, hexanal, ethyl butyrate, octanal, neral, and geranial, and an increase in two undesirable components, furfural and α-terpineol, were observed in addition to other changes. After 2-month storage, an experienced taste panel found a significant difference in stored juices compared to a control sample and a significant preference for the starting control juice. Continued decreases in oil content and ascorbic acid were noted during the 8-month storage period.

Aseptically packaged fruit juices and fruit juice drinks are the fastest growing segment of the fruit beverage industry (Tillotson, 1984). Some of the products have relatively short shelf lives because they undergo flavor changes at supermarket shelf temperatures (21 °C). These changes are particularly noticeable in 100% orange juice that has been packaged aseptically; the flavor changes inhibit the full market potential of this product. There is a need for analytical techniques to provide information on the specific flavor and compositional changes that aseptically packaged fruit juices undergo during storage. From this information, methods can be proposed for inhibiting or retarding such detrimental changes.

Limited studies have been carried out that show changes in a few specific volatile components during storage of orange juice packaged aseptically. Durr et al. (1981) found

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