Syntheses and Sensory Characterization of 5,8,11-Tetradecatrien-2-one Isomers

Akio Kobayashi,* Kikue Kubota, Minoru Iwamoto, and Hiroshi Tamura

Eight geometrical isomers of 5,8,11-tetradecatrien-2-one were selectively synthesized. The values on the ¹H and ¹³C NMR spectra were unambiguously assigned on the basis of the synthetically established stereostructures. By comparing their IR and NMR spectra, the naturally occurring isomers isolated from an aroma concentrate of cooked small shrimps were confirmed to be *all-Z* and 5*E*,8*Z*,11*Z* isomers. A sensory evaluation of the synthesized isomers suggested that the two identical with the natural product had the most acceptable seafood aroma, like that of cooked shrimp and crab, and that these compounds seemed to be important to the flavor of some cooked seafood products.

In the preceding report (Kubota and Kobayashi, 1987), we found two new ketones in the aroma concentrate of cooked small shrimps and estimated their structures as (5*Z*,8*Z*,11*Z*)- and (5*E*,8*Z*,11*Z*)-5,8,11-tetradecatrien-2-one. As these compounds have three double bonds in their structures, there should exist eight geometrical isomers. The determination of the structures of the natural products was mainly based on analyses of their IR and NMR spectra and on their comparison with those of synthesized *all-Z* and *all-E* isomers; however, all possible isomers had not been synthesized at that time.

In this report, we describe the syntheses of all eight isomers of 5,8,11-tetradecatrien-2-one and reconfirm their estimated stereostructure from the collection of spectral data. We also evaluated their aroma characteristics by a sensory test and discusses here the contribution of the newly identified ketones to a seafood aroma like that of cooked small shrimp.

EXPERIMENTAL SECTION

General Synthetic Methods. *(1) Halogenation of 2-Alkenols and 2-Alkynols.* With stirring, 0.1 mol of PBr_3 or PCl_3 was added to an ether solution (150 mL) of 0.3 mol of alcohol and 7 mmol of pyridine during 1.5 h. Stirring at room temperature was continued for 2 h, and the contents were poured on ice. The separated organic layer was washed with 10% $NaHCO_3$ and H_2O and dried on $MgSO_4$. As the obtained unsaturated halogen compounds were too unstable to purify by distillation, they were purified by silica gel column chromatography (developed with hexane–ethyl acetate, 95:5).

(2) Mesylation of 2-Alkenols and 2-Alkynols. The reaction of methylsulfonyl chloride (mesyl chloride) and alcohols in the presence of triethylamine followed the method of Crossland and Servis (1970). All reaction processes were performed below 0 °C, and after the reaction mixture decomposed in ice-cooled water, the mesylate was used to the next process without purification.

(3) Condensation of the Active Halide or Mesylate to an Acetylenic Grignard Complex. This reaction was performed under a dry argon gas atmosphere. Ethylmagnesium bromide was prepared from metal magnesium (0.34 mol) and ethyl bromide (0.34 mol) in 150 mL of dry tetrahydrofuran (THF). To this solution was added a terminal acetylenic compound (0.29 mol) in THF (50 mL) at 35 °C for 0.5 h. After all the ethane gas had evolved, 0.5 g of cuprous iodide was added, before 0.26 mol of halide

or mesylate in THF (50 mL) was added for 30 min. Stirring was continued for 10 h at room temperature, and the contents were decomposed with saturated ammonia. The crude extract was dissolved in 300 mL of methanol containing 1% hydrochloric or sulfuric acid and the resultant mixture stirred for 1 h to remove the protective group. After water was added, the product was extracted with ether and washed with 10% $NaHCO_3$ solution and brine. The product was purified by distillation under reduced pressure.

(4) Partial Reduction of the Triple Bond to a Z Double Bond by Lindlar Catalyst. Palladium on $CaCO_3$ was prepared according to Lindlar's original method (Lindlar and Dubius, 1966). An acetylenic compound was dissolved in hexane (100 mL) with 2 g of catalyst and 5 g of quinoline in the reaction flask of a low-pressure hydrogenation apparatus. To absorb the calculated volume of hydrogen gas, 10–12 h was required.

(5) Lithium Aluminum Hydride (LAH) Reduction of the Triple Bond to an E Double Bond. An acetylenic compound (0.2 mol) was dissolved in 30 mL of THF, and the solution was added to LAH (0.1 mol) in 70 mL of THF under a dry N_2 gas stream at room temperature. Stirring was continued for several hours, and the LAH complex was decomposed with saturated NH_4Cl solution.

(6) Methyl Ketone Synthesis by Condensation with Ethyl Acetoacetate. Ethyl acetoacetate (0.34 mol) was added to 0.33 mol of sodium ethoxide in 150 mL of absolute EtOH. After 1 h of stirring at 40 °C, 0.3 mol of halide or mesylate was added at the same temperature. Stirring was continued overnight, and the mixture was refluxed for 1 h, followed by cooling and acidification with acetic acid, before the product was poured into ice-cooled water. After being extracted with ether, the residue was hydrolyzed with 10% NaOH solution for 1 h at 65 °C. The pH was adjusted to 6.0 with 20% H_2SO_4, and decarboxylation by refluxing for 1 h yielded the methyl ketone.
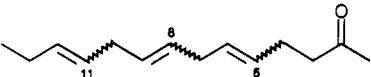
Except for the halide and mesylate, all synthetic intermediates were purified by distillation in vacuo. Small amounts of the final products were purified by preparative gas chromatography.

Instrumental Analyses. The IR spectra were measured as a smear on a sodium chloride plate with a Jasco Model IRA-1 IR spectrometer. ¹H NMR and ¹³C NMR spectra were recorded on a JEOL JN-GX270 (270-MHz) FT-NMR spectrometer. All samples were dissolved in $CDCl_3$ with Me_4Si as an internal standard.

To obtain the low-resolution MS, the GC-MS system was used under the following conditions: 50 m × 0.25 mm (i.d.) FS-WCOT column coated with PEG 20M; helium carrier gas, 1.2 mL/min flow rate, 30:1 split ratio; 60 °C (4 min hold) oven temperature programmed at 2 °C/min up to 180 °C; JEOL DX-300 double-focusing mass spec-

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Table I. Chemical Data and Aroma Characteristics for Isomers of 5,8,11-Tetradecatrien-2-one

isomer	yield, %	M⁺ (m/e) obsd (mmu)[a]	retention index (KI)	aroma characteristics
I (5Z,8Z,11Z)[b]	16	206.1670 (−0.1)	2014	shrimp, crab, shellfish
II (5E,8Z,11Z)	24	206.1630 (−4.1)	2033	shrimp, crab, sea cucumber
III (5Z,8E,11Z)	15	206.1640 (−3.1)	2037	fruity, oily, seafood
IV (5Z,8Z,11E)	15	206.1674 (0.3)	2011	fruity, oily, milk
V (5E,8E,11Z)	15	206.1670 (−0.1)	2041	short-legged clam, seafood
VI (5E,8Z,11E)	14	206.1695 (2.4)	2029	short-legged clam, oily
VII (5Z,8E,11E)	14	206.1651 (−2.0)	2030	fruity, cucumber, white-meat fish
VIII (5E,8E,11E)	13	206.1657 (−1.4)	2033	oily, fishy, dry bonito

[a] Numbers in parentheses are differences from the theoretical m/z value in millimass units. [b] Numbers are the carbon numbers, and Z and E are the arrangements of their atoms in space.

trometer, electron impact ionization at 70 eV.

The purified synthetic final products were injected directly into the same MS to measure the high-resolution m/z value at their molecular ion peak. All MS data were processed by a DA-5000 data processing system.

Sensory Description. This was carried out by a panel of 15 male experts and 10 female assistants who deal with perfumery at Hasegawa Perfumery Inc. Their ages ranged from 20 to 40 years, and the average age was 31. Among the 25 persons, there were 5 smokers. The smelling paper was dipped in a 3–4% ethanol solution of each purified synthetic product and was smelled so that its aroma characteristics could be described. After the preliminary test, 13 expressions listed in Table I were selected for aroma characters and the panelist was requested to choose from those words to express the characteristics of each aroma.

RESULTS AND DISCUSSION

Syntheses of Eight Isomers of 5,8,11-Tetradeca-trien-2-ones. Stereoselective syntheses of polyunsaturated aliphatic alcohols, acetates, or ketones are widely applied in insect pheromone chemistry (Henrick, 1977). The acetylenic compounds were useful as starting materials because of the triple bond, which was easily reducible to a Z or E double bond selectively at any stage of the synthesis and was also the active functional group to make a new C–C bond. The strategy for the syntheses of C_{14} ketones is shown in Figure 1, where the C_{14} carbon chain was divided into one C_5 unit and three C_3 units, each of which started from an acetylenic compound and ethyl acetoacetate. The detailed experimental conditions to prepare each intermediate followed the literature (Henrick, 1977), so that only an outline of the synthetic process is shown in Figure 2.

For the synthesis of *all-Z* isomers, the terminal C_5 unit (2-pentyn-1-ol, 1) was brominated to 1-bromo-2-pentyne, which was then condensed with tetrahydropyranylpropynol (2) in the presence of Grignard reagent and cuprous iodide.

After acidic hydrolysis to remove the protective tetrahydropyranyl group, 2,5-octadiynol (3) was transformed to the corresponding bromide 4, which was condensed to the diethylene ketal of 5-pentyn-2-one (5) under the same condition as with the Grignard complex of 2 to give 5,8,11-tetradecatriyn-2-one (6). Partial hydrogenation of 6 by hydrogen gas and Lindlar catalyst gave the desired (5Z,8Z,11Z)-tetradecatrien-2-one (I), in a good yield. To obtain the 5E,8Z,11Z isomers, 3 was partially hydrogenated to (2Z,5Z)-octadienol (7), which was converted to the bromide (8a), or mesylate (8b) for condensing with 2 to yield (5Z,8Z)-undecadien-2-ynol (9). Lithium aluminum hydride reduced the OH-conjugated (hydroxymethyl)-
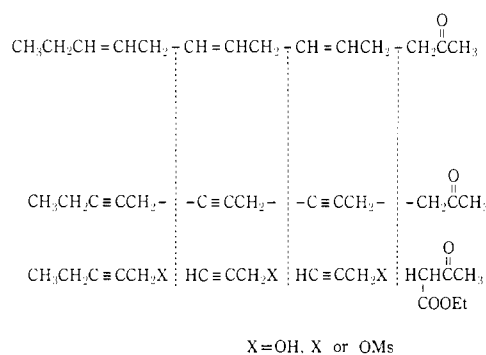

Figure 1. Starting materials to synthesize C_{14} ketones.

acetylene group in 9 to the corresponding E double bond, and the resulting trienol (10a) was converted to the mesylate (10b) and condensed to form an ethyl acetoacetate. Alkaline hydrolysis and decarboxylation under the acidic condition gave (5E,8E,11Z)-tetradecatrien-2-one (II).

Starting from 1, the other six isomers were selectively synthesized by properyl combining the synthetic process just described, i.e., substitution of the hydroxy group with a halo or mesyl group to combine two different acetylenic compounds, selective hydrogenation of the triple bond to a double bond with Lindlar catalyst or lithium aluminum hydride, and introduction of the C_3 methyl ketone unit by condensation with ethyl acetoacetate. The general synthetic processes are summarized in Figure 3, and each yield based on 2-pentynol (1) as the starting material is given in Table I, together with the high-resolution MS data at their molecular ion peaks, their retention indices (Kovats index, KI) obtained under the same gas chromatographic conditions as for GC–MS measurement, and their aroma characteristics, which will be discussed in a later section.

Spectrometric Analyses of Eight Isomers and Their Comparision with Those of Natural Products. As shown in Table I, the difference in KI values was not large enough to identify the natural products directly. Figure 4 is a mass spectrum of the synthetic *all-Z* isomer; however, the other seven isomers showed almost the same spectrum. As discussed in the preceding report, the IR spectra of two natural products differed only at about 960 cm⁻¹, and if the synthetic isomers had E double bonds in the molecule, the IR spectra were very similar, except for the strength of the absorption band at 960 cm⁻¹.

Therefore, an analysis and comparison of the NMR spectra seemed to be more useful for unequivocally identifying the natural products with the synthetic isomers. The ¹H NMR data of eight isomers are summarized in Table II. The most distinctive feature was a chemical shift
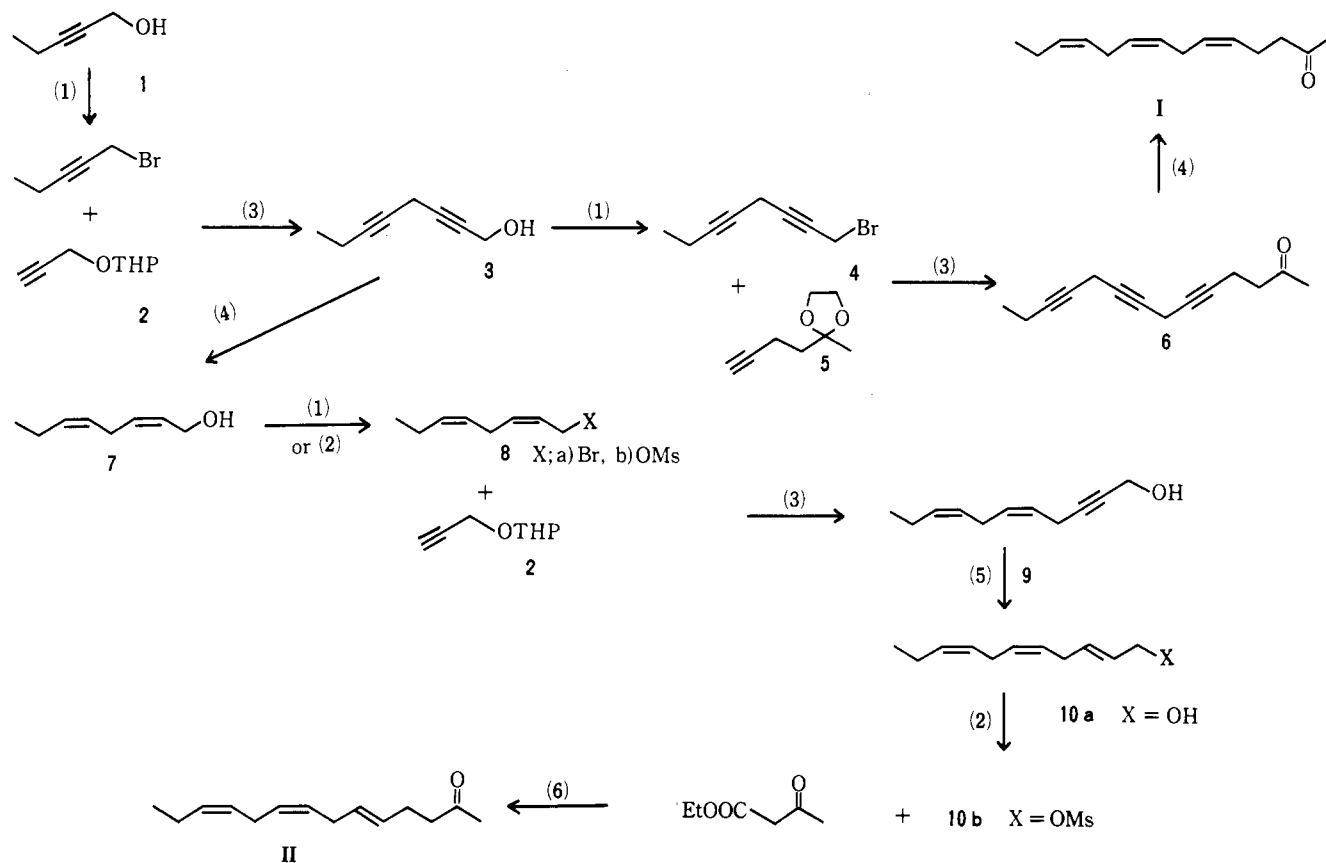
5,8,11-Tetradecatrien-2-one Isomers

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Figure 2. Syntheses of (*all-Z*)- and (5*E*,8*Z*,11*Z*)-tetradecatrien-2-one. Numbers in parentheses correspond to the synthetic methods described in Results and Discussion.

Table II. ^{1}H NMR Data for Eight Isomers of 5,8,11-Tetradecatrien-2-one [CDCl$_3$, δ (*J*, Hz)]

				carbon no. (assignment)			
isomer[a]	14 (C*H$_3$*, 3 H, t)	13 (CH$_3$C*H$_2$*, 2 H, m)	1 (COC*H$_3$*, 3 H, s)	4 (C*H$_2$*CH=, 2 H, m)	3 (C*H$_2$*C=O, 2 H, t or m)	7,10 (=CHC*H$_2$*CH=, 4 H, m)	5,6,8,9,11,12 (=C*H*, 6 H, m)
I	0.97 (7.6)	2.05–2.13	2.14	2.33–2.38	2.49 (t, 7.3)	2.78–2.85	5.26–5.45
II	0.97 (7.6)	2.01–2.09	2.13	2.23–2.33	2.46–2.52 (m)	2.70–2.80	5.25–5.50
III	0.96 (7.6)	1.98–2.10	2.13	2.27–2.35	2.45–2.51 (m)	2.70–2.78	5.27–5.52
IV	0.97 (7.4)	1.95–2.07	2.14	2.28–2.38	2.46–2.55 (m)	2.70–2.87	5.28–5.53
V	0.96 (7.6)	1.99–2.10	2.13	2.22–2.31	2.49 (t, 7.8)	2.63–2.70, 2.70–2.76	5.28–5.52
VI	0.96 (7.3)	1.95–2.06	2.13	2.23–2.30	2.49 (t, 7.3)	2.66–2.68	5.31–5.57
VII	0.97 (7.3)	1.96–2.06	2.13	2.27–2.37	2.48–2.51 (m)	2.74–2.78, 2.66–2.69	5.32–5.54
VIII	0.97 (7.6)	1.96–2.07	2.14	2.23–2.34	2.50 (t, 7.3)	2.67–2.70	5.34–5.53

[a] Refer to Table I.

Table III. ^{13}C NMR Data for Eight Isomers of 5,8,11-Tetradecatrien-2-one (CDCl$_3$, δ)

isomer[a]	C-14	C-13	C-12	C-10	C-7	C-4	C-3	C-2	C-1	C-5,6,8,9,11
I	14.3	20.6	132.1	25.6	25.7	21.7	43.5	208.2	29.9	127.0, 127.9, 128.2, 128.6, 129.1
II	14.2	20.5	132.0	25.4	30.3	26.7	43.4	208.3	29.9	127.0, 127.4, 128.8, 129.3
III	14.2	20.4	132.2	30.3	30.2	21.6	43.4	208.1	29.8	126.8, 128.3, 128.5, 128.8, 129.0
IV	13.8	25.5	132.5	30.3	25.5	21.6	43.4	208.2	29.9	126.9, 128.0, 128.3, 129.1
V	14.7	20.8	132.6	30.7	35.9	27.1	43.8	208.6	30.3	127.2, 128.9, 129.4, 129.7, 130.0
VI	13.8	25.5	132.5	30.3	30.2	26.7	43.4	208.2	29.9	127.6, 127.8, 128.6, 128.7, 129.3
VII	13.8	25.5	132.7	35.5	30.3	21.6	43.4	208.1	29.9	127.3, 128.3, 128.5, 128.8, 129.3
VIII	13.9	25.6	132.8	35.6	35.5	26.8	43.5	208.5	30.0	127.4, 128.9, 129.0, 129.7

[a] Refer to Table I.

of the protons adjacent to the double bond. For example, peaks of the C-4, C-7, C-10, and C-13 protons of the *all-Z* isomer I shifted to a lower field than those of the *all-E* isomer II. As the other six isomers had both *Z* and *E* double bonds in their molecules, the corresponding methylene protons show an intermediate value between those of I and II. This tendency can be more clearly recognized by the analyses of ^{13}C NMR data. As shown Table III, the chemical shift of C-13 adjacent to the *Z*

double bond was 20.5 ppm (δ vlaue); however, a lower shift to 25.5 ppm was observed for the C-13 to *E* double bond. A lower shift of 5 ppm was also observed for the C-4 atom by changing the adjacent double bond from *Z* to *E* configuration. Carbon atoms at the 7- and 10-positions were sandwiched by two double bonds, and their chemical shifts were greatly affected by these two configurations; i.e., a value of 26 ppm was assigned to the methylene carbon between *Z* and *Z*, 36 ppm between *E* and *E*, and the in-
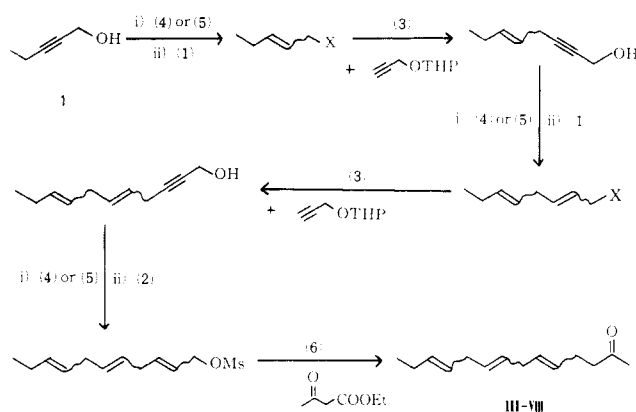
Figure 3. General synthetic routes to the geometrical isomers of 5,8,11-tetradecatrien-2-one (III–VIII).
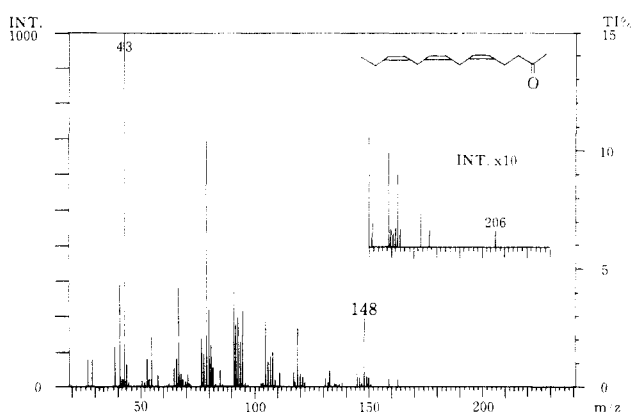

Figure 4. Mass spectrum of synthetic (5Z,8Z,11Z)-tetradeca-trien-2-one.

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; (2Z,5E,8Z)-CH₃(CH₂CH=CH)₂CH₂OMS, 117606-26-5; (2Z,5Z,8E)-CH₃(CH₂CH=CH)₃CH₂OMS, 117606-27-6; (2E,5E,8Z)-CH₃(CH₂CH=CH)₃CH₂OMS, 117606-28-7; (2E,5Z,8E)-CH₃(CH₂CH=CH)₃CH₂OMS, 117606-29-8; (2Z,5E,8E)-CH₃(CH₂CH=CH)₃CH₂OMS, 117606-30-1; (2E,5E,8E)-CH₃(CH₂CH=CH)₃CH₂OMS, 117606-31-2; CH₃COCH₂COOEt, 141-97-9; (5E,8Z)-CH₃(CH₂CH=CH)₂CH₂C≡CCH₂OH, 117606-32-3; (5Z,8E)-CH₃(CH₂CH=CH)₂CH₂C≡CCH₂OH, 117606-33-4; (5E,8E)-CH₃(CH₂CH=CH)₂CH₂C≡CCH₂OH, 101339-91-7; (Z)-CH₃CH₂CH=CHCH₂C≡CCH₂OH, 117606-34-5; (E)-CH₃CH₂CH=CHCH₂C≡CCH₂OH, 101339-87-1.

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Analyses of Steam Distillates and Aqueous Extracts of Smokeless Tobacco

Edmond J. LaVoie, Patricia Tucciarone, Mark Kagan, John D. Adams, and Dietrich Hoffmann*

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 Research 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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