Formation of 2-Alkyl-(2*H*)-thiapyrans and 2-Alkylthiophenes in Cooked Beef and Lamb

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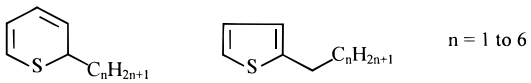
2-Alkyl-(2*H*)-thiapyrans and 2-alkylthiophenes have been identified in the volatiles of cooked beef and lamb. The quantities of both groups of compounds were higher in the meat of animals fed lipid supplements high in *n*−3 polyunsaturated fatty acids. 2-Alkyl-(2*H*)-thiapyrans were formed when (*E*,*E*)-2,4-dienals (C_6−C_{11}) and hydrogen sulfide were heated at 140 °C for 30 min. This confirmed their proposed route of formation in cooked meat from lipid-derived aldehydes and hydrogen sulfide; the latter was produced from the degradation of cysteine, via the Maillard reaction. The mass spectra and NMR spectra of these thiapyrans are reported for the first time. Although 2-alkyl-(2*H*)-thiapyrans were found to have only low odor potency, the reactions by which they are formed may have important implications for meat flavor. These reactions may remove potent aroma compounds and their intermediates from meat, thus modifying the overall aroma profile.

Keywords: *Flavor; 2-alkyl-(2H)-thiapyrans; 2-alkylthiophenes; meat; Maillard reaction; lipids; alkadienals*

INTRODUCTION

Volatiles formed from the interactions of lipids and their degradation products with intermediates from the Maillard reaction have been reported in a number of cooked foods (Whitfield, 1992). The interaction between lipids and the Maillard reaction may affect flavor in three ways: (i) lipid−Maillard reaction products may have flavor characteristics; (ii) compounds with low odor thresholds derived from lipid oxidation, for example, unsaturated aldehydes, may react with Maillard intermediates, reducing their contribution to rancid and other odors; and (iii) Maillard intermediates, for example, ammonia and hydrogen sulfide, can react with lipid-derived volatiles, reducing their availability for the formation of cooked flavors.

Although compounds from lipid−Maillard interactions have been reported in model systems, relatively few of these compounds have been isolated from foods (Whitfield, 1992; Farmer and Mottram, 1994; Elmore et al., 1997). Two groups of compounds, which may arise from lipid−Maillard interactions, are 2-alkyl-(2*H*)-thiapyrans (**1**) and 2-alkylthiophenes (**2**), containing six



1 **2**

carbon atoms or more. Such thiophenes have been reported in pork (*n* = 2−8, Werkhoff et al., 1993), beef (*n* = 2, 4, 5, 8, Wilson, 1973; *n* = 2−8, Min et al., 1979; *n* = 5, Persson and von Sydow, 1973), lamb (*n* = 6, Sutherland and Ames, 1995), and poultry (*n* = 3, Crawford and Kretsch, 1976; *n* = 4−5, Tang et al., 1983; *n* = 5, Noleau and Toulemonde, 1986; *n* = 6, Noleau and Toulemonde, 1988; *n* = 3,Werkhoff et al., 1993), as well as fruit, nuts, vegetables, seafood, coffee, and fungi (Nijssen et al., 1996). 2-Methylthiophene has also been found in cooked meat and other heated foods but is likely to be derived from the action of hydrogen sulfide on 2-methylfuran or from thiamin decomposition (van der Linde et al., 1979).

Only one thiapyran has been reported in food: 2-pentylthiapyran in cooked beef heart (Farmer and Mottram, 1994). However, 2-alkyl-(2*H*)-thiapyrans with side chains from C_2 to C_7, along with 2-alkylthiophenes with side chains from C_3 to C_8, were isolated from cysteine/ribose/lecithin reaction mixtures (Farmer and Mottram, 1990). 2-Pentylthiapyran and 2-hexylthiophene were identified as major products when 2,4-decadienal and hydrogen sulfide were reacted in aqueous solution at pH 8 (van den Ouweland et al., 1989). In all of the above cases it was assumed that the double bonds in the thiapyran ring were in the 3- and 5-positions, although this assumption was not confirmed by nuclear magnetic resonance spectroscopy (NMR).

In this paper, six 2-alkylthiophenes and six 2-alkyl-(2*H*)-thiapyrans have been identified and quantified in the headspace volatiles of cooked beef and lamb. All of the thiapyrans have been synthesized and their identities confirmed by gas chromatography−mass spectrometry (GC-MS) and proton NMR. The work reported in this paper arises from studies on the quality of beef and lamb from cattle and sheep fed diets that attempted to modify the polyunsaturated fatty acid (PUFA) composition (Elmore et al., 1999, 2000).

EXPERIMENTAL PROCEDURES

Materials. (*E*,*E*)-2,4-Hexadienal, (*E*,*E*)-2,4-heptadienal, (*E*,*E*)-2,4-decadienal, and (*E*,*E*)-2,4-undecadienal were obtained from Lancaster Synthesis, Morecambe, U.K.; (*E*,*E*)-2,4-octadienal and (*E*,*E*)-2,4-nonadienal were from Aldrich Chemical Co.,

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Gillingham, U.K. Hydrogen sulfide was obtained in a pressurized lecture bottle from S.I.P. Analytical, Basildon, U.K.

Animals and Diets. Beef and lamb were obtained from feeding trials carried out to examine the effect of diet on the PUFA composition of ruminant muscle. The trials involved a control diet containing palm oil, a linseed supplement, a fish oil supplement, and a combined fish oil/linseed supplement. These three diets resulted in increased levels of α-linolenic acid (18:3*n*−3), eicosapentaenoic acid (20:5*n*−3), and docosahexaenoic acid (22:6*n*−3) in the muscle from the animals fed with the unsaturated lipid supplements, compared with the control. The cattle were all Charolais steers (Elmore et al., 1999), and the sheep were of two types, Soay and Suffolk × Lleyn crosses (Elmore et al., 2000).

Preparation of Aroma Extracts. Samples of beef and lamb (M. longissimus lumborum) trimmed of fat were cooked in an autoclave at 140 °C for 30 min. Aroma volatiles were collected on Tenax TA, using the method described by Elmore and Mottram (1998). During collection the sample was held at 60 °C for 1 h while nitrogen, at 40 mL/min, swept the volatiles onto a glass-lined, stainless steel trap (105 mm × 3 mm i.d.) containing 85 mg of Tenax TA (Scientific Glass Engineering Ltd., Milton Keynes, U.K.). A standard (100 ng of 1,2-dichlorobenzene in 1 μL of hexane) was added to the trap at the end of the collection, and excess solvent and any water retained on the trap were removed by purging the trap with nitrogen at 40 mL/min for 5 min. For each treatment samples from five animals were analyzed.

Preparation of Reaction Mixtures. A saturated solution of hydrogen sulfide was made by bubbling the gas through deionized water at 1 °C in a fume hood. *Caution: Hydrogen sulfide is toxic.* An aliquot (20 mL) was placed in a thick glass ampule, containing 2 mmol of straight-chain, saturated aldehyde. The ampules were sealed, then heated for 120 min at 140 °C in an autoclave, and allowed to cool.

The reaction mixtures were each extracted three times with 10 mL of redistilled *n*-pentane (Analar grade, Fisons, Loughborough, U.K.) and stored at −18 °C.

Flash Chromatography. Each extract was concentrated to 1 mL and then separated on a flash chromatography column (30 cm × 1.4 cm i.d.; Sigma-Aldrich Co. Ltd., Poole, U.K.) containing silica gel 60 (40−63 μm for column chromatography; Merck Ltd., Poole, U.K.), using redistilled *n*-pentane (150 mL) as the eluting solvent. Chromatography was performed under nitrogen pressure at a flow rate of 5 mL/min. Fractions containing thiapyrans were analyzed by GC-MS. They were then concentrated to dryness and dissolved in deuterated chloroform ($CDCl_3$; Aldrich Chemical Co.). Anhydrous sodium sulfate (Merck Ltd.) was added to the $CDCl_3$ as a drying agent.

GC-MS. All analyses were performed on a Hewlett-Packard 5972 mass spectrometer, fitted with an HP5890 Series II gas chromatograph and a G1034C Chemstation. The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 μA. The mass spectrometer was scanned from *m*/*z* 29 to 400 at 1.9 scans/s for the cooked meat samples and the reaction mixture pentane extracts; for the pure 2-alkyl-(2*H*)-thiapyrans, it was scanned from *m*/*z* 10 to 200 at 3.1 scans/s.

A series of *n*-alkanes (C_6−C_{22}) was analyzed, for each set of chromatographic conditions, to obtain linear retention index (LRI) values for the meat aroma components and the reaction products.

GC-MS of Cooked Meat. A CHIS injection port (Scientific Glass Engineering Ltd., Milton Keynes, U.K.) was used to thermally desorb the meat volatiles from the Tenax trap onto the front of a 5% phenylsiloxane/95% methylsiloxane fused silica capillary column. During the desorption period of 5 min, the oven was held at 0 °C. After desorption, the oven was heated at 40 °C/min to 40 °C and held for 2 min before heating at 4 °C/min to 280 °C.

Quantification of the 2-alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans in the cooked meat was based on the relationship between their peak areas and that of the 1,2-dichlorobenzene internal standard, obtained from the total ion chromatograms, using a response factor of 1. This method of quantification is suitable for comparing treatments, but absolute values are only approximate because the mass spectrometer is more sensitive to compounds that fragment to a greater degree.

GC-MS of Reaction Mixture Products. Pentane extracts (1 μL) were injected in split mode at 250 °C (split ratio 50:1) onto a CP-Sil 8 CB low-bleed/MS fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness; Chrompack, London, U.K.). The oven was held at 60 °C for 2 min before heating at 15 °C/min to 280 °C.

Mass spectra and LRI values for the pure 2-alkyl-(2*H*)-thiapyrans were obtained under similar conditions but with splitless injection, the splitter opening after 1 min, and the GC oven temperature increased at 4 °C/min. LRI values were also obtained on a BP20 column (50 m × 0.32 mm i.d.; Scientific Glass Engineering Ltd.), using a similar temperature program but with a final temperature of 250 °C.

NMR Spectroscopy. A Bruker AMX 400 instrument with a 400 MHz operating frequency and a 9.4 T magnet was used to obtain ^{1}H NMR spectra of the pure thiapyrans. A solution of the thiapyran in $CDCl_3$ was placed in a 5 mm tube with a 5 mm probe. One hundred and sixty scans with 6 s repetition were measured.

RESULTS AND DISCUSSION

2-Alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans were found in all of the meat samples, although effects of diet and species were evident (Table 1). Quantities of both thiophenes and thiapyrans were higher in the meat of animals fed PUFA supplements. This corresponded to higher quantities of *n*−3 PUFAs found in the muscles of these animals (Elmore et al., 1999, 2000). The associated higher levels of lipid breakdown products, such as 2,4-dienals, in the cooked meat with increased levels of PUFAs may be responsible for the larger quantities of thiophenes and thiapyrans. The 2-alkylthiophenes eluted from the GC column almost 100 LRI units earlier than their isomeric 2-alkyl-(2*H*)-thiapyrans.

There were also higher levels of thiophenes and thiapyrans in lamb compared with beef, although quantities of PUFAs did not differ appreciably between the species. This could be due to higher levels of hydrogen sulfide, which have been reported in the muscle of sheep meat compared with beef (Kunsman and Riley, 1975). Hydrogen sulfide is a breakdown product of cysteine and glutathione, both present at higher levels in lamb compared with beef (Macy et al., 1964a,b).

To confirm the identities of these compounds, 2,4-alkadienals were reacted with hydrogen sulfide in aqueous solution. 2-Alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans were produced in all of these reactions. However, the degree of reaction decreased as the chain length of the dienal increased. The quantities of alkylthiapyrans recovered were much greater than those of the equivalent thiophenes; thiapyrans were present at levels of up to 100 times higher than thiophenes in the pentane extracts. A small amount of 2-methyl-(2*H*)-thiapyran was also found in an (*E*,*E*)-2,4-hexadienal/hydrogen sulfide reaction mixture after storage at room temperature for 24 h. In a reaction mixture containing (*E*,*E*)-2,4-nonadienal, ammonium sulfide, and acetoin, a major product was tentatively identified as 2-pentylthiophene (Elmore and Mottram, 1997). However, we now know that the compound was 2-butyl-(2*H*)-thiapyran.

Mass spectra for the 2-alkyl-(2*H*)-thiapyrans are listed in Table 2. All of the mass spectra have a base peak at *m*/*z* 97, due to loss of the alkyl side chain from

Table 1. Quantities of 2-Alkyl-(2*H*)-thiapyrans and 2-Alkylthiophenes (Nanograms per 100 g of Meat) Found in the Headspace Volatiles of Cooked Lamb and Beef from Animals Fed Dietary Lipid Supplements[a]

		lamb								beef Charolais			
		Suffolk				Soay							
compound	LRI[b]	control	linseed	fish/linseed	fish	control	linseed	fish/linseed	fish	control	linseed	fish/linseed	fish
2-alkylthiophenes													
ethyl	868	13	14	32	32	12	13	18	23	5[a]	6[a]	8[a]	15[b]
propyl	961	2	3	4	4	2	3	4	3	tr	tr	tr	tr
butyl	1065	2	4	5	4	2	3	5	4	–	tr	tr	tr
pentyl	1169	4	3	6	4	3	3	5	4	tr	tr	tr	tr
hexyl	1274	4	4	7	5	4	3	7	6	tr	tr	tr	tr
heptyl	1379	tr	tr	2	2	tr	tr	1	1	–	–	–	–
2-alkyl-(2*H*)-thiapyrans													
methyl	912	3	3	5	6	tr	tr	3	4	–	–	–	–
ethyl	1019	22	59	87	131	19	15	56	65	3[a]	6[a,b]	9[b]	14[c]
propyl	1117	2[a]	4[a]	10[a,b]	15[b]	tr	tr	7	8	–	tr	tr	2
butyl	1221	tr	tr	2	2	tr	tr	tr	tr	–	–	–	–
pentyl	1328	5	4	7	8	4	tr	7	5	–	tr	tr	tr
hexyl	1436	–	–	tr	tr	–	–	tr	tr	–	–	–	–

[a] Means in the same row with different superscripts are significantly different ($P < 0.05$); means are from five replicate samples; tr, <1 ng of headspace of 100 g sample; –, <0.1 ng in the headspace of 100 g sample. [b] Linear retention index on a CP-Sil 8 CB low-bleed/MS column.

Table 2. Mass Spectral and NMR Data of 2-Alkyl-(2*H*)-thiapyrans Formed by the Reaction of (*E*,*E*)-2,4-Alkadienals and Hydrogen Sulfide

	LRI			
(2*H*)-thiapyran	CP-Sil 8 CB	BP20	mass spectral data [*m*/*z* (rel intensity)][a]	^{1}H NMR data (400 MHz, $CDCl_3$, TMS)
2-methyl	915	1290	97, **112** (25), 45 (12), 111 (11), 39 (9), 77 (8), 98 (6), 53 (6), 99 (5), 51 (5), 69 (4), 58 (4), 50 (4), 27 (4), 78 (3)	δ 1.31 (d, ^3J = 7 Hz, 3H) 3.45 (m, ^3J = 6, 7 Hz, 1H), 5.57 (dd, ^3J = 10, 6 Hz, 1H), 5.96 (dd, ^3J = 6, 10 Hz, 1H) 6.14 (dd, ^3J = 9, 6 Hz, 1H), 6.20 (d, ^3J = 9 Hz, 1H)
2-ethyl	1020	1394	97, **126** (14), 45 (9), 39 (7), 98 (6), 99 (5), 53 (5), 27 (4), 77 (3), 69 (3), 51 (3), 111 (2), 84 (2), 65 (2), 58 (2)	δ 0.98 (d, ^3J = 7 Hz, 3H) 1.68 (q, ^3J = 7, 7 Hz, 2H), 3.45 (m, ^3J = 6, 7 Hz, 1H), 5.55 (dd, ^3J = 10, 6 Hz, 1H), 5.96 (dd, ^3J = 6, 10 Hz, 1H) 6.12 (dd, ^3J = 9, 6 Hz, 1H), 6.18 (d, ^3J = 9 Hz, 1H)
2-propyl	1117	1487	97, **140** (12), 98 (7), 45 (7), 39 (6), 99 (5), 53 (4), 27 (4), 11 (3), 77 (3), 51 (3), 84 (2), 69 (2), 67 (2), 65 (2)	δ 0.89 (d, ^3J = 7 Hz, 3H), 1.2–1.7 (bm, 2 × 2H), 3.33 (m, ^3J = 6, 7 Hz, 1H), 5.55 (dd, ^3J = 10, 7 Hz, 1H), 5.96 (dd, ^3J = 6, 10 Hz, 1H), 6.12 (dd, ^3J = 9, 6 Hz, 1H), 6.18 (d, ^3J = 9 Hz, 1H)
2-butyl	1221	1597	97, **154** (9), 98 (7), 45 (6), 99 (5), 39 (5), 111 (3), 77 (3), 53 (3), 41 (3), 27 (3), 84 (2), 65 (2), 51 (2), 29 (1)	δ 0.89 (d, ^3J = 7 Hz, 3H), 1.2–1.7 (bm, 3 × 2H), 3.33 (m, ^3J = 6, 7 Hz, 1H), 5.55 (dd, ^3J = 10, 7 Hz, 1H), 5.97 (dd, ^3J = 6, 10 Hz, 1H), 6.12 (dd, ^3J = 9, 6 Hz, 1H), 6.18 (d, ^3J = 9 Hz, 1H)
2-pentyl	1329	1700	97, **168** (8), 98 (7), 99 (5), 45 (5), 39 (5), 41 (4), 27 (4), 77 (3), 53 (3), 29 (3), 111 (2), 65 (2), 51 (2), 84 (1)	δ 0.86 (d, ^3J = 7 Hz, 3H) 1.2–1.7 (bm, 4 × 2H), 3.33 (m, ^3J = 6, 7 Hz, 1H), 5.55 (dd, ^3J = 10, 6 Hz, 1H), 5.96 (dd, ^3J = 6, 10 Hz, 1H), 6.12 (dd, ^3J = 9, 6 Hz, 1H), 6.18 (d, ^3J = 9 Hz, 1H)
2-hexyl	1434	1804	97, **182** (7), 98 (7), 99 (5), 45 (4), 53 (3), 41 (3), 39 (3), 27 (3), 11 (2), 77 (2), 29 (2), 67 (1), 65 (1), 51 (1)	δ 0.85 (d, ^3J = 7.2 Hz, 3H) 1.2–1.7 (bm, 5 × 2H), 3.33 (m, ^3J = 6, 7 Hz, 1H), 5.55 (dd, ^3J = 10, 6 Hz, 1H), 5.96 (dd, ^3J = 6, 10 Hz, 1H) 6.12 (dd, ^3J = 9, 6 Hz, 1H), 6.18 (d, ^3J = 9 Hz, 1H)

[a] First ion listed is the base peak; molecular ion is in boldface type.

the six-membered ring. As the base peak is at *m*/*z* 97, the two double bonds in the ring are likely to be at the 3- and 5-positions; otherwise, cleavage of the alkyl chain would occur α to the double bond, giving a strong ion at *m*/*z* 111.

The mass spectra of 2-alkylthiophenes are very similar to those of 2-alkyl-(2*H*)-thiapyrans, the main difference being that the molecular ions of 2-alkylthiophenes are up to 3 times greater than those of their isomeric 2-alkyl-(2*H*)-thiapyrans. Additionally, as the chain length of these compounds increases, the intensity of the *m*/*z* 98 ion increases in 2-alkylthiophenes but remains the same in 2-alkyl-(2*H*)-thiapyrans. This implies that the five-membered thiophene ring is more stable than the six-membered (2*H*)-thiapyran ring, facilitating the loss of a neutral alkene from the side chain to give positively charged 2-methylthiophene. Charge location is more likely to occur on the relatively polar thiophene ring rather than on the nonpolar side chain. Hence, increased loss of the $C_{n-1}H_{2n-2}$ alkene occurs in the 2-alkylthiophenes, as the length of the side chain increases.

The NMR spectra (Table 2) of all six 2-alkyl-(2*H*)-thiapyrans showed five signals, caused by the protons attached to the five carbon atoms in the six-membered (2*H*)-thiapyran ring. Figure 1 shows which signals corresponded to the carbon atoms in the ring. For each signal, the chemical shifts were almost identical for all of the 2-alkyl-(2*H*)-thiapyrans; only the signal furthest upfield changed significantly, decreasing in frequency as the length of the alkyl substituent increased. It was determined that this signal was due to the protons in the methylene group in the 2-position of the ring. The low chemical shift, relative to the other four signals, indicated the absence of a double bond; it was a multiplet, and the decrease in shift with increasing side-

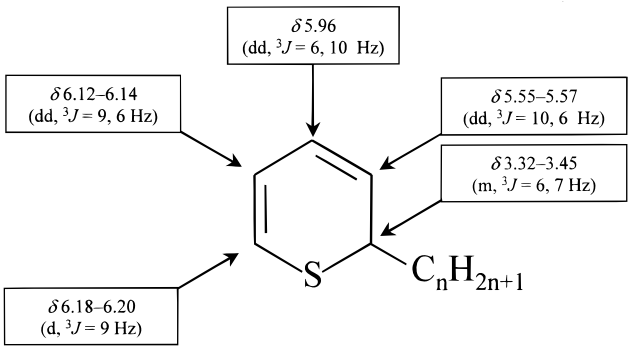

Figure 1. NMR data (chemical shift, degree of coupling, coupling constant) for the protons in the six-membered ring of 2-alkyl-(2*H*)-thiapyrans.

chain length confirm that this carbon was in the 2-position. The signal at δ 6.18−6.20 was a doublet, and it was due to the proton of the carbon in the 6-position. The other three signals were double doublets, and their positions could be assigned by comparison of their coupling constants with those of the protons on the 2- and 6-positions of the ring.

The methylene groups in the side chains of the thiapyrans were not resolved and were present between δ 1.20 and 1.70. The terminal methyl group moved upfield with increasing chain length. It had a chemical shift of 1.31 in 2-methyl-(2*H*)-thiapyran, decreasing to 0.85 in 2-hexyl-(2*H*)-thiapyran.

In all of the GC-MS chromatograms small quantities of pairs of compounds eluted with LRI values between 15 and 20 units higher than those of the 2-alkyl-(2*H*)-thiapyrans. Both compounds had the same mass as the 2-alkyl-(2*H*)-thiapyran. Their mass spectra were similar to each other but differed from the mass spectrum of the 2-alkyl-(2*H*)-thiapyran. Both of the unknown compounds had a strong *m/z* 111 and a strong $(M - 1)^{\bullet+}$ ion; both of these ions were weak in the 2-alkyl-(2*H*)-thiapyran. The $(M - 1)^{\bullet+}$ ion was of similar intensity to the molecular ion in both of the unknown compounds, the latter having a greater intensity than the molecular ion of the corresponding 2-alkyl-(2*H*)-thiapyran. The two unknown compounds were tentatively identified as 2-alkyl-(4*H*)-thiapyrans and 2-alkyl-(6*H*)-thiapyrans, both of which would fragment to give a strong *m/z* 111 ion, as discussed earlier. Insufficient quantities of these compounds were present to allow their NMR spectra to be obtained.

Van den Ouweland et al. (1989) discussed the reaction mechanism for the formation of 2-alkylthiapyrans. They suggested that additional compounds, 2-alkyldihydrothiapyranthiols, could be formed in the reaction mixtures from the addition of hydrogen sulfide across one of the double bonds in the thiapyran. In the present work, three peaks were present at trace levels in the chromatograms of all of the reaction mixtures, with mass spectra that could be explained by this addition. For each compound the base peak was at $(M - 33)^{\bullet+}$, with major peaks at $M^{\bullet+}$, $(M - 67)^{\bullet+}$, *m/z* 45, and *m/z* 85.

The aromas of all six of the alkylthiapyrans were weak and thiophene-like in quality; the lower molecular weight thiapyrans possessed an unpleasant garlic-like note. Because of their weak odor intensities, it is unlikely that any of these compounds contribute directly to cooked meat aroma. However, the reactions by which they are formed may be of importance in cooked meat flavor because such reactions may modify the profile of aroma compounds, as discussed under Introduction. For example, the reaction of 2,4-alkadienals with hydrogen sulfide, to form 2-alkyl-(2*H*)-thiapyrans, leads to decreases in the concentrations of these potent aroma compounds while forming a compound with low aroma significance.

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