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Received for review September 1, 1971. Accepted September 15, 1972.

Synthetic Precursors of Flavor Compounds with a Thiol Group

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Flavoring agents possessing a thiol group are usually difficult to apply in foodstuffs due to their volatility and instability. A search was made for a suitable protection of the thiol group to yield a precursor which releases the flavoring material during heating. The *O-tert*-alkyl thiocarbonates, particularly the *O-tert*-butyl thiocarbonate, appeared to be satisfactory. These esters hydrolyze in an aqueous medium at elevated temperatures to give the thiol of interest. The thiocarbonates of nine flavoring thiols were prepared and investigated.

Thiols are usually very powerful flavoring agents and are therefore generally used in concentrations less than 1 ppm. However, their application is limited as they are relatively volatile and highly reactive. They are, for example, easily oxidized to disulfides, and elimination of hydrogen sulfide may also occur. As a result of these two factors, thiol flavoring compounds rapidly disappear from food products during storage and heat treatment.

An illustrative example of a thiol flavoring compound is 1-methylthio-ethanethiol, which was isolated from beef broth in this laboratory (Brinkman *et al.*, 1972). When added to a foodstuff this compound is only perceived immediately after its addition, and then usually too strongly, since it is very volatile and unstable in an aqueous medium. Natural precursors of 1-methylthio-ethanethiol were found to be alanine, methionine, and cysteine (Schutte and Koenders, 1972). However, yields of formation of the flavoring compound from these precursors are very low and many other aromas are formed.

The addition of a nonvolatile derivative of the flavoring substance which slowly decomposes during heating, thus releasing the compound of interest in reasonable yields, would overcome the above drawbacks. It was therefore decided to investigate the slow formation of thiols from derivatives having a heat-labile thiol-protecting group. The protecting group increases the molecular weight and consequently decreases the volatility of the material so that the derivative should have little flavoring properties itself. The derivative should be sufficiently stable to be prepared, purified, and stored in a foodstuff, and finally, it should decompose during heating of the product to give the desired thiol.

In the present investigation we prepared some esters of thiols that are known to be important aroma compounds. The release of thiols during heating of the esters in an aqueous medium was conceived to take place as represented below.

$$R'-S-\overset{\overset{X}{\|}}{C}-R'' + H_2O \xrightarrow{\Delta} R'-SH + HO-\overset{\overset{X}{\|}}{C}-R'' \qquad (1)$$

$X = O, S$

The efficiency of release of the thiols from the precursors was determined by analytical methods. The thiol investigated primarily was 1-methylthio-ethanethiol [$R' = CH_3SCH(CH_3)$]. Later the study was extended to other flavoring substances with thiol groups. As examples of this important class, 1-butanethiol ($R' = C_4H_9$) and 2-butanethiol [$R' = CH(CH_3)C_2H_5$] were selected. Heterocyclic thiols, particularly tetrahydrofuran-, dihydrofuran-, and furanthiols, have been found to contribute to roasted meat odor (van den Ouweland and Peer, 1968); therefore this type of compound was also investigated. Finally, furfurylmercaptan, an important flavor compound contributing to the aroma of coffee (Staudinger and Reichstein, 1928) was

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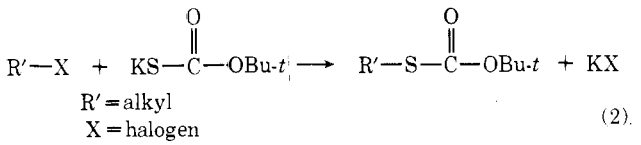
included in our study. The various esters are represented in Table I.

EXPERIMENTAL SECTION

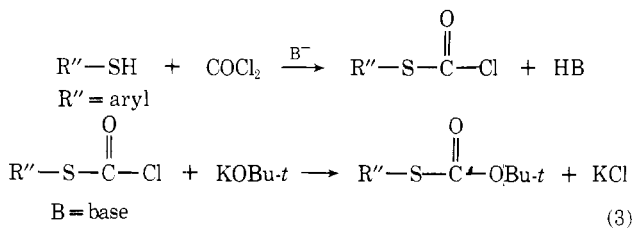
Synthesis of Precursors. Thiol precursors were prepared by two routes. The alkyl series was prepared by reaction of the corresponding halogeno compounds with the potassium salt of *tert*-butyl monothiocarbonate.



R′ = alkyl
X = halogen

(2)

Derivatives **14** and **15** were prepared from the thiols by treatment with phosgene to give the chlorothioformates which were then allowed to react with *tert*-butoxide.



B = base

(3)

Another tertiary alkyl substituent [*i.e.*, $C(CH_3)_2C_3H_7$] was used in a few examples. The potassium salt of *O-tert*-butyl monothiocarbonate could be obtained in quantitative yield by reaction of solid potassium *tert*-butoxide and gaseous carbonyl sulfide (Hirschbind, 1934). The sodium salt of the *tert*-hexyl monothiocarbonate was prepared from 2-methyl-2-pentanol, sodium hydride, and carbonyl chloride. Two typical examples are given in detail. The physical and spectroscopic data of the compounds are given in Table II.

tert-Butyl S-1-Butyl Thiocarbonate (**9**). To a stirred suspension of 17.2 g (0.1 mol) of potassium *O-tert*-butyl thiocarbonate in 100 ml of acetone was added 6.9 g (0.05 mol) of 1-bromobutane. The mixture was brought to reflux temperature, cooled, and filtered, and the solvent was evaporated under reduced pressure. Fractional distillation yielded 7.2 g (76%) of compound **9**.

tert-Butyl S-Phenyl Thiocarbonate (**15**). 17.7 g (0.102 mol) of phenylthiocarbonyl chloride (Rivier, 1907) was added to a precooled (−20°) suspension of 11.9 g (0.106 mol) of potassium *tert*-butoxide in 150 ml of tertrahydrofuran. The mixture was stirred at room temperature for 1 hr and then filtered. Evaporation of the solvent under reduced pressure gave an oil from which 5.09 g (23%) of **15** was obtained by distillation.

Thiol Formation from the Precursors. About 10 mg of each precursor was boiled from 15 min to 1 hr in 1 l. of an aqueous solution of 1 g of monosodium glutamate, 2 g of casein hydrolysate, 6 g of salt, 1 g of NaH_2PO_4, and 2 g of hardened vegetable fat at pH 5.5. This medium is a crude imitation of a soup. Nitrogen was swept over the surface of the water and led successively through a condenser, a trap at −20°, and a trap at −196°. The contents of the second trap were analyzed by gas chromatography on a Hewlett-Packard 5750 instrument. The 1.5 m × 2 mm glass column was packed with 10% Carbowax 20M–1% Apiezon on 80–100 mesh Diatoport S and was temperature programmed from 50 to 200° at 4° per min. In most cases the thiol formed was identified by its odor and by its retention time, which had been previously established for the pure compound. In some cases the reaction products were collected from the gas chromatographic effluent and identified by mass spectrometry. In some experiments the tertiary alcohol was detected by this method.

Acute Toxicity Tests. In order to have an impression as to whether or not addition of the thiocarbonates to food would present a toxicological hazard, LD_{50} tests were performed on mice with two representative compounds, *i.e.*, compounds **5** and **16**. The tests were performed at the Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, England.

RESULTS AND DISCUSSION

Table III summarizes the relative efficiency of the release of the thiols from the precursors, as estimated from the amount of thiol found in the −196° trap.

The thioacetate **1** was too stable, as were the dithiocarbonates **2** and **4**. On the other hand, the *tert*-butyl ester **3** was too unstable. As it had been reported that monothiocarbonates are more stable than the corresponding dithiocarbonates (O'Connor and Nace, 1953), we hoped that the *O-tert*-butyl monothioester **5** would have just the right degree of stability. Indeed, it was found that the pure compound was perfectly stable when heated to 130°, whereas in boiling aqueous medium slow degradation took place. In aqueous solution at pH 2, the formation of 1-methylthio-ethanethiol proceeded at a far greater rate than at pH 5.5. At pH 8 the formation of thiol was somewhat slower than at pH 5.5.

Usually the hydrolysis of thio esters is base catalyzed (Houben-Weyl, 1955). We found that 1-methylthio-ethanethiol is formed from **1** and **2** in KOH–alcohol in high yields. Another mechanism of dissociation of mono- and dithio esters is the Chugaev reaction (Hine, 1962), a four-center reaction which takes place irrespective of medium and phase. Compound **5**, however, is stable in pure form at elevated temperatures. Its dissociation appears to be acid catalyzed. The following mechanism is therefore proposed.

Table I. Thiol Esters Prepared for This Study

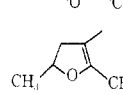
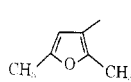
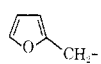
Compd	R′	R″	X
1		$-CH_3$	O
2		$-OC_2H_5$	S
3		$-OC(CH_3)_3$	S
4	$CH_3SCH(CH_3)$	$-OCH(CH_3)_2$	S
5		$-OC(CH_3)_3$	O
6		$-OCH(CH_3)_2$	O
7		$-OC(CH_3)_2C_3H_7$	O
8	C_4H_9-	$-OC(CH_3)_2C_3H_7$	O
9	C_4H_9-		
10	$C_2H_5CH(CH_3)$		
11	$OH(CH_2)_2-$		
12			
13		$-OC(CH_3)_3$	O
14			
15			
16			

Table II. Physical Constants and Spectrometric Data of Thiol Esters

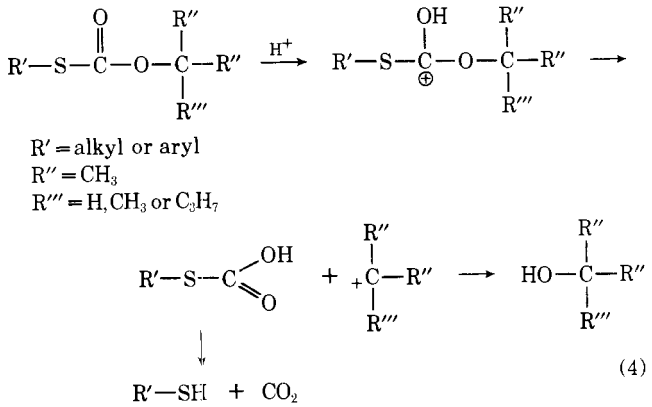
Compd	bp °C (mm)	$n^{20}D$	ms (*m/e*)	ir, cm^{-1}	nmr,[a] δ, ppm
1	70–74 (9)	1.5172	150, 107, 103, 75, 59, 47, 45, 43	2980, 2920, 1690, 1450, 1375, 1356, 1132, 1108, 1060, 950, 730, 700, 625 (neat)	1.62 (d, 3), 4.52 (q, 1), 2.12 (s, 3), 2.30 (s, 3)
2	76 (0.25)	1.5695	196, 150, 136, 75, 60, 59, 47, 45	2980, 2920, 1446, 1374, 1218, 1148, 1111, 1043, 696 (neat)	1.46 (t, 3), 4.62 (q, 2), 1.72 (d, 3), 4.68 (q, 1), 2.22 (s, 3)
3	*b*	*b*	210, 150, 108, 107, 75, 60, 59, 47, 45, 43		1.67 (d, 3), 4.66 (q, 1), 2.20 (s, 3), 1.72 (s, 9)
4	71–73 (0.15)	1.5513		2980, 2920, 1700, 1448, 1388, 1375, 1225, 1090, 1035, 902, 695 (neat)	1.43 (d, 6), 5.74 (m, 1), 1.73 (d, 3), 4.67 (q, 1), 2.22 (s, 3)
5	68 (0.55)	1.4875	208, 152, 151, 108, 75, 74, 60, 59, 56, 47, 45, 41, 39	2982, 2930, 1719, 1702, 1453, 1399, 1374, 1207, 1130, 1060, 858, 840, 700 (CCl_4)	1.54 (s, 9), 2.22 (s, 3), 1.67 (d, 3), 4.30 (q, 1)
6	62–64 (0.1)	1.4914	194, 107, 103, 75, 60, 59, 47, 45, 43	2980, 2920, 1708, 1444, 1384, 1373, 1155, 1095, 1055, 847 (neat)	1.25 (d, 6), 5.01 (m, 1), 1.62 (d, 3), 4.32 (q, 1), 2.14 (s, 3)
7	*c*	*c*	236, 108, 85, 75, 69, 59, 56, 47, 45, 43, 41	2963, 2937, 2879, 1708, 1446, 1387, 1370, 1180, 1130, 1058, 953, 850, 839 (CCl_4)	0.96 (t, 3), 1.2–2.0 (m, 4), 1.46 (s, 6), 2.17 (s, 3), 1.65 (d, 3), 4.28 (q, 1)
8	63–65 (11)	1.4555		2960, 2930, 2870, 1710, 1465, 1457, 1385, 1369, 1180, 1130, 850, 840, 675 (neat)	0.91 (t, 6), 1.42 (s, 6), 1.2–2.0 (m, 8), 2.71 (m, 2)
9	82–84 (20)	1.4511	190, 146, 90, 59, 58, 57, 56, 55, 47, 43, 41	2980, 2960, 2932, 2877, 1716, 1702, 1456, 1392, 1367, 1246, 1197, 1127, 856, 835 (CCl_4)	0.92 (t, 3), 1.1–1.8 (m, 4), 1.44 (s, 9), 2.72 (t, 2)
10	71–73 (15)	1.4482	190, 117, 101, 90, 59, 57, 55, 43, 41	2963, 2926, 2874, 1715, 1699, 1453, 1391, 1367, 1197, 1120, 838 (neat)	0.95 (t, 3), 1.4–2.0 (m,2), 1.28 (d, 3), 3.2 (m, 1), 1.43 (s, 9)
11	93–95 (0.3)	1.4788	192, 119, 92, 74, 57, 41, 39	3390, 2980, 2940, 1702, 1396, 1371, 1203, 1130, 1050, 857, 840 (neat)	1.82 (m, 2), 3.58 (t, 2), 2.83 (t, 2), 3.26 (s, 1), 1.43 (s, 9)
12	*d*		218, 85, 84, 74, 73, 57, 56, 55, 45, 43, 41	2980, 2930, 2865, 1717, 1702, 1450, 1392, 1368, 1244, 1196, 1128 (CCl_4)	1.17 (d, 3), 3.4–4.0 (m, 3), 1.5–2.7 (m, 2), 3.1 (m, 1), 1.37 (s, 9)
13	*e*			2981, 2928, 2861, 1720, 1697, 1453, 1392, 1377, 1369, 1226, 1197, 1122, 958, 838 (CCl_4)	1.38 (d, 3), 4.65 (m, 1), 1.85 (t, 3), 2.37, 2.80 (m, 2), 1.48 (s, 9)
14	*f*	1.4970	228, 128, 127, 113, 85, 57, 43	2980, 2925, 1730, 1580 1450, 1397, 1371, 1230, 1200, 1125, 1070, 990, 925, 840 (neat)	
15	70–74 (0.2)	1.5280	210, 137, 110, 109, 57, 51, 50, 41, 39	2980, 1723, 1476, 1440, 1392, 1368, 1196, 1120, 1087, 1022, 836, 748, 688 (neat)	7.2–7.5 (m, 5), 1.45 (s, 9)
16	115–119 (9)	1.4960	214, 158, 137, 81, 57, 53, 41		6.20 (m, 2), 7.26 (m, 1), 3.97 (d, 2), 1.45 (s, 9)

[a]Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Numbers in parentheses are integrated values. [b]Decomposes at room temperature. [c]Isolated by column chromatography (Al_2O_3, activity I), eluent chloroform. [d]Isolated by preparative glc. [e]Isolated by thin-layer chromatography (silica gel), eluent chloroform (R_f 0.78). [f] As footnote *e*. Eluent benzene–pentane (1:3, v/v, R_f 0.48).

Table III. Estimated Release of Thiol from 10 mg of Precursor in 1 l. of Boiling Aqueous Medium (pH 5.5)

Precursor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Thiol formation[a]	—	—	**	—	++	+	+++	++	+	+	+	++	+	+	++	+++

[a]+++, high; ++, moderate; +, low; —, none; **, unstable.



R′ = alkyl or aryl
R″ = CH_3
R‴ = H, CH_3 or C_3H_7

(4)

The formation of a tertiary carbonium ion is the driving force of this hydrolysis. Replacement of the carbonyl by a thiocarbonyl group appears to enhance the rate of dissociation, since **4** was found to be very unstable.

CONCLUSIONS

O-tert-Butyl monothiocarbonate thiol esters are slowly hydrolyzed in hot aqueous medium to give the corresponding thiols. Probably partly due to their lower volatility, the precursors have threshold values which are generally more than ten times higher than those of the thiols. They can therefore be added to foodstuffs below their threshold values and still generate the thiols in organoleptically discernible amounts. Examples of some applications are described in a pending patent (van der Heijden and Schutte, 1971).

The *O-tert*-butyl moiety may be replaced by any other tertiary alcohol group. This makes the purification after separation more difficult, since the volatility of the thiocarbonate decreases with increasing size of the alcohol grouping. On the other hand, this diminished volatility may be advantageous for the release of the thiol, as the thiocarbonate is evaporated from the food with even more difficulty and therefore has more chance to hydrolyze. Indeed, the formation of thiols from *O-tert*-hexyl esters **7** and **8** proceeded even more readily than from the corresponding *O-tert*-butyl esters **5** and **9**.

The LD_{50} values of compounds **5** and **16** for mice were found to be 3.67 and 0.58 ml/kg, respectively. These values do not preclude usage of these compounds in food.

ACKNOWLEDGMENTS

The authors are indebted to E. B. Koenders and T. L. van Orden for technical assistance, and S. B. Tjan, J. A. M. Arends and W. Stek, for measuring the NMR, IR, and mass spectra. The contribution of R. Munday, who established the LD_{50} values, is also gratefully acknowledged.

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Received for review September 7, 1972. Accepted October 30, 1972.

Physicochemical Changes in Some Pakistani Mango Varieties during Storage Ripening

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Some physicochemical changes in four popular Pakistani mango varieties during storage ripening have been studied. Acidity decreases during ripening with the simultaneous increase in reducing sugars. Formol value, nonreducing sugars, after reaching a peak after 4 days, start decreasing. The same 12 free amino acids have been identified and estimated in all the varieties. Both total and individual amino acids increase in concentration in the early ripening period and then decrease. Malda variety is the richest in proteins, amino acids, and sugars and possesses the least acidity. The organoleptic maturity coincides closely with the peak values of amino acids and sugars after 4–8 days during ripening.

Mango is known as the king of table fruits and is universally popular due to its pleasant flavor and healthful dietetic qualities. From the point of view of acreage and production, it is one of the most outstanding of all Pakistani fruits. Many varieties are available in the market, and endeavors are being made to develop newer varieties. These varieties differ in flavor, nutritional qualities, and rate of spoilage under similar storage conditions. A large fraction of the total production is spoiled due to over-ripening and ultimate fermentative breakdown of carbohydrates.

A study of the changes occurring during ripening will yield useful information which would serve a dual purpose. It would help the agriculturists to develop new varieties with better overall qualities by cross-breeding and it would help the food technologists to estimate maturity and develop better preservation techniques.

Studies on changes in chemical composition during ripening of some varieties of mangoes of the Indo-Pakistan subcontinent have been reported (Basu *et al.*, 1947; Cheema *et al.*, 1950; Lely *et al.*, 1940; Srivastaga, 1953; Wahab and Khan, 1954). This work was undertaken to study the

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