Combinatorial Approach to Flavor Analysis. 1. Preparation and Characterization of a *S*-Methyl Thioester Library

Jeffrey A. Khan,[†] Laurence Gijs,[‡] Céline Berger,[§] Nathalie Martin,[§] Geneviève Piraprez,[‡] Henry E. Spinnler,[§] Evgeny N. Vulfson,*[,†] and Sonia Collin[‡]

Department of Macromolecular Sciences, Institute of Food Research, Earley Gate, Whiteknights Road, Reading RG6 6BZ, U.K., Unité de Brasserie et des Industries Alimentaires, Université Catholique de Louvain, Place Croix du Sud 2 Boîte 7, Louvain-la-Neuve, B-1348, Belgium, and Institut National de la Recherche Agronomique, F-78850 Thiverval-Grignon, France

A new method for the "one-pot" synthesis of *S*-methyl thioesters has been developed by reacting methyl chlorothiolformate with carboxylic acids. The resulting "flavor library" contained all the intended thioesters and a single major impurity, identified by GC−MS as *S*,*S*-dimethyldithiocarbonate. Quantification of individual compounds present in the library was performed by GC analysis using two independent methods of detection, SCD and FID. It was shown that apart from *S*-methyl thioacetate (0.8 mol %), molar concentrations of other thioesters varied in a relatively narrow range from 4.2 mol % for *S*-methyl thiopropionate to 14.1 mol % for *S*-methyl thiohexanoate. In general, medium chain *S*-methyl thioesters were present in slightly higher molar concentrations than those prepared from short or long chain carboxylic acids. This variation was attributed to partial loss of the most volatile components during extraction and the lower reactivity of higher homologues. The library was used for the characterization of some physicochemical parameters of thioesters. In particular, lipophilicity coefficients (log k_w) and thioester retention in 10, 20, and 33% triolein (used as a model lipid phase) were determined directly by reverse-phase HPLC and extrapolated from the respective data. This analysis illustrates that substantial information can be generated using a library containing a relatively large number of compounds in effectively the same way as is necessary for the analysis of a single sample.

Keywords: *Combinatorial synthesis; flavor library; thioester; cheese; lipid retention; lipophilicity coefficient*

INTRODUCTION

Isolation, identification, and characterization of flavor compounds in food is a notoriously difficult and painstaking task. The same very much applies to the organoleptic analysis of flavors, where the utmost care in experimental design and multiple repetitions are required to ensure the statistical validity of conclusions and to minimize both the inevitable subjectivity associated with the use of flavor descriptors and variations in the overall organoleptic response of panel judges to a particular compound. Therefore it would be attractive to intensify the process of flavor characterization and/or analysis by handling "arrays" of compounds rather than individual flavorants in a manner not too dissimilar, perhaps, to the combinatorial synthesis/deconvolution employed in the pharmaceutical industry for the acceleration of the drug discovery process (Terrett et al., 1995).

In general, the combinatorial approach is based on a parallel or one-pot synthesis of so-called "libraries" consisting of a mixture of homologous compounds, which are often combined in all possible permutations and then tested for biological activity using an appropriate screening technique. If a multicomponent library shows a "hit" in the biological screen (e.g., inhibition of a target enzyme or receptor), a series of "deconvolution" syntheses are undertaken to identify the "lead" and, finally, to establish the precise structure of the inhibitor. This can be achieved, for example, by the preparation of smaller, partial libraries to determine which one of them contains the active compound and repeating the experiment with even smaller libraries until the most potent structure(s) are identified. The size of the library may vary from 10 to 100 000 compounds depending on the sensitivity of the screening and the nature of target, as well as the chemistry and synthetic methodology employed. Numerous recent reviews summarize the preparation of functionally diverse combinatorial libraries, synthetic methods, and the techniques used for biological screening (Chaiken and Janda, 1996; Gordon and Kerwin, 1998; Obrecht and Villalgordo, 1998; Terrett et al., 1995; Wilson and Czarnik, 1997). Undoubtedly combinatorial chemistry is a powerful tool for the discovery of new drugs and biomolecules with specific functions, but to the best of our knowledge, it has not yet been applied in the food industry.

In this and an accompanying paper (Berger et al., 1999), we report our initial results on the preparation and characterization of flavor libraries. The main objective of this work was to develop and validate the methodology and, if successful, to assess the scope for potential applications. Thioesters were selected as a

* Corresponding author. Telephone: 44 118 9357000. Fax: 44 118 9267917. E-mail: jenya.vulfson@bbsrc.ac.uk.
[†] Institute of Food Research.
[‡] Université Catholique de Louvain.
[§] Institut National de la Recherche Agronomique.

10.1021/jf9902043 CCC: $18.00
Published on Web 07/20/1999

suitable model target in this investigation for the following reasons: (i) Thioesters have been identified by classical methods as one of the known classes of compounds which are formed in maturing cheeses and contribute to their characteristic aroma. In particular, they are believed to be one of the principle aroma components in extracts from smear-ripened semihard cheeses and Gouda (Bosset and Gauche, 1993; Dumont et al., 1974; Dumont et al., 1976; Parliment et al., 1982). (ii) The presence of thiols and carboxylic acids (the chemical precursors of thioesters) in every cheese matrix suggests that they are likely to be involved in the aroma of other varieties too. (iii) Thioesters have exceptionally low odor thresholds (Cuer et al., 1979) and occur in cheese matrices in very small amounts. The isolation of trace quantities of relatively labile compounds is notoriously difficult, while the individual synthesis of all possible structures for the purpose of identification is simply impractical. In this paper we describe the synthesis and preliminary characterization of a library of *S*-methyl thioesters.

MATERIALS AND METHODS

Chemicals. Carboxylic acids, *n*-decylamine, dichloromethane, triethylamine, calcium hydride, 4-(dimethylamino)pyridine (DMAP), methyl chlorothiolformate, (*S*-methyl chlorothiocarbonic acid), anhydrous sodium sulfate, sodium hydrogen carbonate, and citric acid were obtained from Aldrich Chemical Co. Ltd., (Dorset, U.K.). 3-Morpholinopropanesulfonic acid (MOPS) and uracil were purchased from Sigma Chemicals (Dorset, U.K.). *S*-Methyl thioacetate and *S*-methyl thiobutanoate were supplied by Lancaster Synthesis Ltd. All solvents were of the highest purity available and distilled over calcium hydride prior to use.

Synthesis of a Combinatorial Library of *S*-Methyl Thioesters. A 1 mmol sample of each carboxylic acid (acetic, propionic, *n*-butanoic, *n*-pentanoic, *n*-hexanoic, *n*-octanoic, *n*-decanoic, lauric, myristic, palmitic, and stearic) was dissolved in dry dichloromethane (50 mL) containing dry triethylamine (1.34 g, 13.24 mmol) and DMAP (0.13 g, 1.06 mmol) with stirring under dry nitrogen. After this clear solution was cooled to 0 °C, methyl chlorothiolformate (1.34 g, 12.12 mmol) in dry dichloromethane (20 mL) was added dropwise over 20 min. The pale yellow solution was stirred for a further 1 h at 0 °C followed by incubation at room-temperature overnight. The reaction mixture was washed with ice-cold 5% aqueous sodium hydrogen carbonate (50 mL) and 5% aqueous citric acid (50 mL). The organic layer was dried over anhydrous sodium sulfate and filtered and the solvent removed in vacuo (10 mmHg) at 0 °C to yield a canary yellow oil (1.02 g) which was stored as a semisolid at 4 °C. A 1 μL aliquot of the oil was dissolved in dichloromethane (600 μL) for GC analysis.

GC and GC−MS Analysis. This was performed under two different sets of conditions as described below. Evaluation of the retention index of each compound was performed by comparison of their retention time with that of the corresponding alkane.

GC Analysis with FID. This was performed using an HP 5890 GC (Split) System with a BPX-5 capillary column (12 m × 0.3 mm; film thickness = 0.33 μm) connected to a FID and using helium as a carrier gas at a flow rate of 0.7 mL min^{-1}. Samples were injected using a split/splitless injector, split ratio 1/100. The injection temperature was set to 40 °C, and the detector was maintained at 370 °C. The oven temperature was programmed from 40 to 200 °C at 10 °C min^{-1} and then to 360 °C at 25 °C min^{-1}.

For the quantification of individual compounds in the library, calibration curves were constructed by triplicate injections of 10, 20, 50, and 100 ng quantities of *S*-methyl thioester standards (Berger et al., 1999). A linear regression of these data permitted the calculation of response factors to determine the concentration of other *S*-methyl thioesters present in the library.

GC Analysis with SCD. This was performed using a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250 °C and opened after 0.5 min. Analysis of sulfur compounds was performed using a 50 m × 0.32 mm, wall-coated open tubular (WCOT) apolar CP-SIL 5 CB capillary column (film thickness, 1.2 μm) connected to a sulfur chemiluminescence detector (Sievers, Model 355 SCD) and Shimadzu CR3A integrator. An initial oven temperature of 40 °C was maintained for 4 min and then programmed to rise from 40 to 132 °C at 2 °C min^{-1} followed by 132−250 °C at 10 °C min^{-1}. The final temperature was then held for 45 min. Helium carrier gas was used at a flow velocity of 32.0 cm s^{-1} (flow rate=1.0 mL min^{-1}). Air and hydrogen flow were maintained at 40 and 100 mL min^{-1}, respectively in the 800 °C combustion room. The air flow rate in the ozone generator was 6 psi and a vacuum of 150−275 Torr was applied to the entire system.

EI Mass Spectroscopy. Mass spectra were recorded at 70 eV on a 5988 quadrupole mass spectrometer connected to a Hewlett-Packard Model 5890 gas chromatograph equipped with a splitless injector and the above-described columns. Spectral recording was automatic throughout elution using HP 59970C software. The compounds were identified on the basis of peak enhancement by two co-injections of authentic standards and comparison with the NBS/EPA/NIH mass spectra database (this has a record of the *S*-methyl thioesters of *n*-butanoic, *n*-hexanoic, and *n*-octanoic acid).

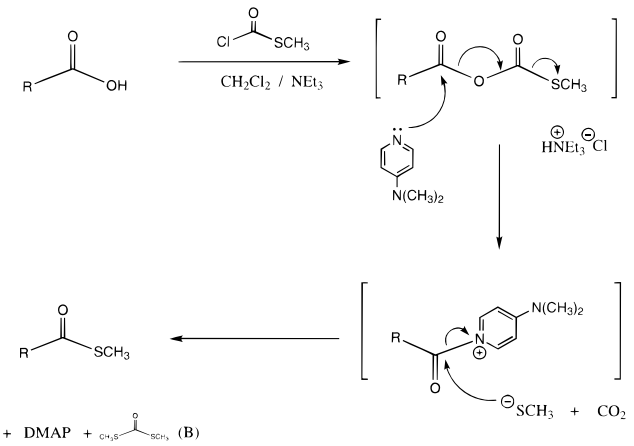
Lipophilicity Measurements. These were performed by reverse-phase (RP)−HPLC equipped with a Waters 510 isocratic pump and a Waters WISP 710 B autosampler. The detector was a Perkin-Elmer LC 75 operating at 230 nm. The Guard-Pak insert was packed with μBondapak C18, particle size 10 μm (Waters) and the column (25 cm × 4 mm) was prepacked with LiChrosorbRP-18, particle size 10 μm (Merck). A Digital 380 PC equipped with the Waters 840 acquisition program (version 6.0) was used as an integrator for peak recording and the calculation of retention times. The mobile phase was comprised of methanol (30−70%) and 10 mM MOPS buffer (pH 7.4) containing *n*-decylamine (0.2% v/v) which was used as a masking agent to eliminate silanophilic interactions (El Tayar et al., 1985a). The methanol/aqueous solution was filtered with a Millipore HAWP filter (0.45 μm). Retention times (t_r) were measured at room temperature at a flow rate of 1.5 mL min^{-1}. The column dead time (t_o) was determined with uracil. The capacity factor is defined as $k = (t_r - t_o)/t_o$. Log k for 100% water (log k_w) was linearly extrapolated from results obtained for different mobile phase compositions (El Tayer et al., 1985 a,b).

RESULTS AND DISCUSSION

To produce a flavor library that is amenable to analysis of individual components, a synthetic procedure is required which can provide all the intended compounds in a similar quantity. Although the acylation of sodium thiomethoxide with an acid chloride in dichloromethane is a simple and efficient method for the preparation of individual thioesters (Berger et al., 1999), distillation under reduced pressure is necessary to remove impurities and produce organoleptically pure compounds. This approach, as well as many other methods of thioester synthesis, is therefore unsuitable for the preparation of a "balanced" library due to the difficulty of distilling a mixture of products with a large range of boiling points. Thioesters are also prone to thermal degradation at elevated temperatures, which makes the distillation of mixtures containing higher molecular weight homologues unattractive.

For this reason, an alternative strategy was investigated which would not involve the requirement for distillation of the library. It is known that reaction of

Scheme 1. Synthesis of a *S*-Methyl Thioester Library from Carboxylic Acids and Methyl Chlorothiolformate in the Presence of DMAP



alkyl chloroformates with carboxylic acids in the presence of DMAP can be used for the preparation of esters via the intermediate formation of a mixed carboxylic carbonic anhydride (Kim et al., 1985), and hence we envisaged that a *S*-methyl thioester may be produced in an analogous manner using methyl chlorothiolformate (Scheme 1). This was indeed found to be the case, and a typical GC chromatogram of a *S*-methyl thioester library prepared with 11 homologous carboxylic acids is depicted in Figure 1a. Even brief examination of this chromatogram showed that the library was very "clean" and contained the expected 11 thioester products. Importantly, there was no requirement to distill this mixture due to the formation of a single major impurity (*S*,*S*-dimethyldithiocarbonate (peak B), a byproduct formed by the side reaction of methanethiolate with methyl chlorothiolformate) and a second minor impurity (peak A) which was not identified.

We then proceeded with the assignment of peaks in the flavor library and confirmation of the structure of the individual compounds present. The peaks were provisionally assigned on the basis of retention index and then analyzed by GC−MS. EI mass spectra showed the expected fragmentation of each *S*-methyl thioester due to the loss of the thiomethyl group, and the structure of the predicted impurity (*S*,*S*-dimethyldithiocarbonate, peak B) was also confirmed on the basis of the parent molecular ion (m/z $[M]^+ = 122$).

The quantification of individual compounds in the library was performed using two independent methods. First, the library was reanalyzed by GC equipped with a sulfur chemiluminescence detector (SCD) (Figure 1b) and the concentrations of thioesters and impurities present were determined directly (Table 1). GC analysis with SCD confirmed the presence of one major (peak B) and two minor impurities (peaks A and C respectively). The very minor peak C, which appeared between *S*-methyl thiopalmitate (**C16**) and *S*-methyl thiostearate, (**C18**) was observed by SCD, but not FID (Figure 1a) presumably due to the much higher sensitivity of the former detector. The relative peak area of impurity B by SCD (Figure 1b) also appeared larger than that obtained by FID (Figure 1a) due to the presence of two sulfur atoms in this library component.

The results presented in Table 1 suggest that apart from *S*-methyl thioacetate (**C2**), the molar concentrations of other thioesters varied in a relatively narrow range from 4.2 (*S*-methyl thiopropionate, **C3**) to 14.1 mol % (*S*-methyl thiohexanoate, **C6**) with a maximum value obtained for medium chain (**C5**, **C6**) acids. This can be attributed to partial loss of the most volatile components during workup of the library (the boiling point of *S*-methyl thioacetate (**C2**) is 97 °C) and the slightly lower reactivity of longer chain fatty acids used in the thioester synthesis. Clearly it was possible to adjust the initial concentration of each carboxylic acid in the reaction mixture to provide a *S*-methyl thioester library containing each component in a very similar quantity. However, we decided against this strategy as it was not clear from the outset whether such an adjustment would be necessary for the characterization and organoleptic assessment of flavor libraries.

An alternative method to determine the relative concentrations of individual components in the library was also investigated. We reasoned that, although the SCD was ideally suited for the analysis of *S*-methyl thioesters, it would not be generally useful for the quantification of flavor compounds which did not contain sulfur. As the prime objective of this study was to develop general methodology, it was of interest to demonstrate that conventional FID could also be used to accurately determine the concentration of each library component and in addition, to assess how this method compared with that of SCD. To this end, calibration graphs for several *S*-methyl thioesters (**C3**, **C4**, **C6**, **C10**, and **C14**) (Berger et al., 1999) were constructed, and the concentration of each compound in the library was determined directly. A linear regression of these data enabled the extrapolation of response factors for other homologues in the library, and these values, together with respective peak areas, were used to determine their relative concentrations (Table 2). In most cases, the calculated and experimentally determined values determined by the two methods were in good or reasonable agreement.

Once the library was characterized with regard to the relative concentration of individual compounds present, the utility of this approach to assess the behavior of *S*-methyl thioesters in food matrices was investigated. In particular, the library was used to determine lipophilicity coefficients (log k_w) and the retention of *S*-methyl thioesters by triolein which acted as a model lipid phase. This information is useful to assist in formulation of food products with optimal flavor release. The lipophilicity coefficients were determined by reverse phase HPLC which was eluted with 30, 40, 50, 60, and 70% of methanol (see Materials and Methods). Only *S*-methyl thioesters of carboxylic acids containing 6 carbon atoms or less were analyzed in this experiment due to the excessively long retention times of the higher homologues. However, the linear relationship between lipophilicity and carbon chain length obtained for the first five thioesters (**C2**−**C6**) in the library (Figure 2) enabled the calculation of log k_w values for all the compounds of interest and the data obtained was used to determine the retention of each *S*-methyl thioester in 10, 20, and 33% triolein (Table 3). As expected, the higher homologues (carbon chain length >8) were virtually quantitatively retained by triolein (reciprocal retention is ≤1) even at the lowest oil concentration of 10%. This analysis clearly illustrated that a wealth of valuable information could be generated using a flavor library containing a relatively large number of compounds in effectively the same way (and time!) as is necessary to analyze a single sample.

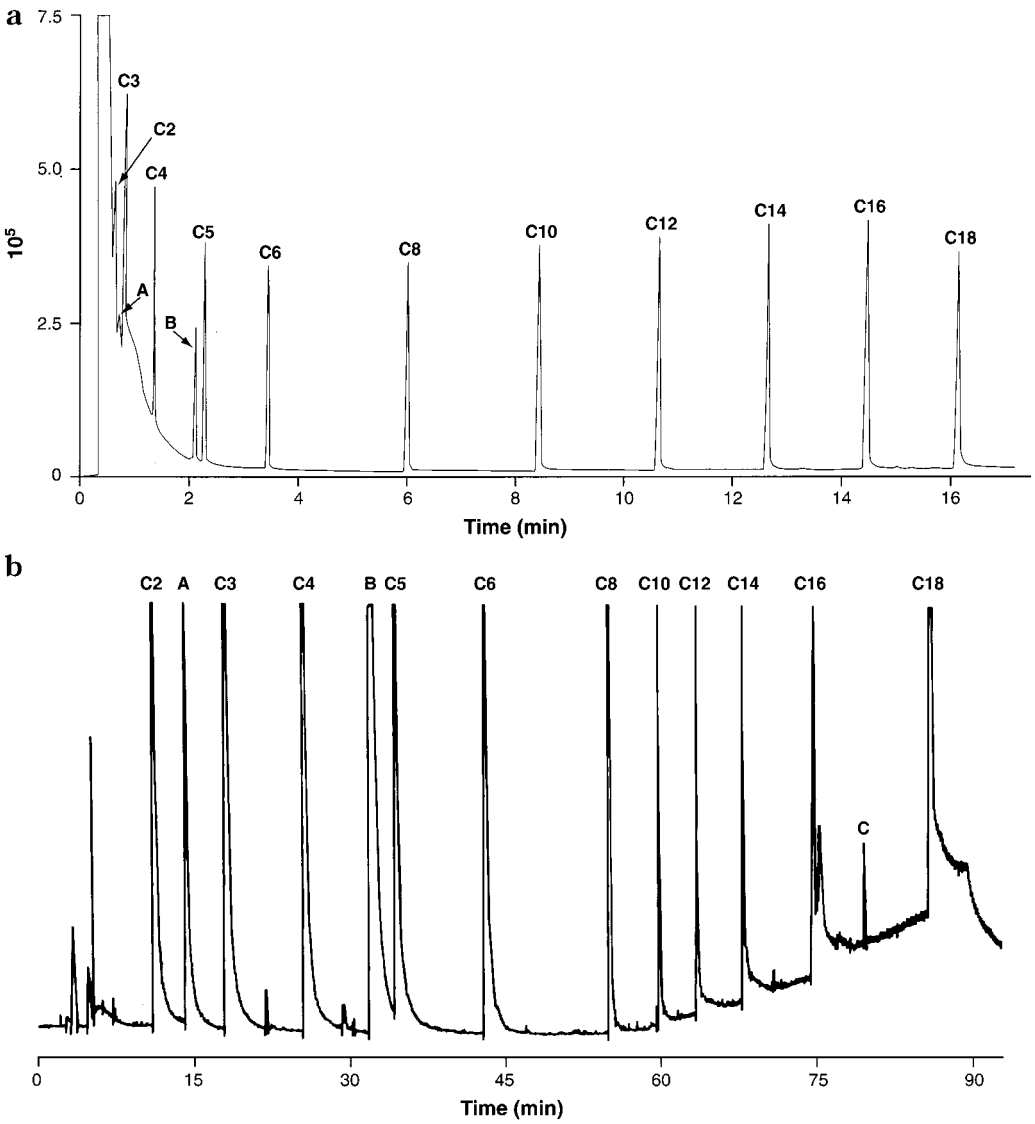


Figure 1. (a) GC analysis using FID for the assessment of purity of a *S*-methyl thioester library. (b) Quantification of the components present in a *S*-methyl thioester library by GC analysis and SCD.

Table 1. Quantification of Individual Components in the *S*-Methyl Thioester Library Using SCD

library component	rel molar concn by SCD[a] (mol %)	mol wt (g mol^{-1})	rel molar concn × mol wt (g)	rel wt (%)
C2	0.8	90	72.0	0.4
impurity A	0.8			
C3	4.2	104	436.8	2.4
C4	8.5	118	1003.0	5.6
impurity B	12.6	122	1537.2	8.6
C5	12.5	132	1650.0	9.2
C6	14.1	146	2058.6	11.5
C8	10.0	174	1740.0	9.7
C10	7.5	202	1515.0	8.5
C12	7.1	230	1633.0	9.1
C14	7.7	258	1986.6	11.1
C16	7.9	286	2259.4	12.6
C18	6.4	314	2009.6	11.2

[a] Calculated values are normalized to compensate for the presence of two sulfur atoms in impurity B.

Table 2. Comparison of SCD and FID in the Determination of the Concentration of Individual *S*-Methyl Thioesters in the Flavor Library

	SCD		FID	
library component	rel [thioester] (mol %)	[thioester][a] (g L^{-1})	rel [thioester] (mol %)	[thioester][b] (g L^{-1})
C3	4.2	24.0	5.8	32.8
C4	8.5	56.0	8.4	53.9
C5	12.5	92.0	9.7	69.5*
C6	14.1	115.0	10.4	82.2
C8	10.0	97.0	14.1	121.7*
C10	7.5	85.0	11.1	104.5
C12	7.1	91.0	11.6	117.9*
C14	7.7	111.0	10.8	117.5
C16	7.9	126.0	10.1	118.2*
C18	6.4	112.0	7.9	97.7*

[a] Calculated thioester concentration using weight percent values obtained by SCD (Table 2). [b] Values calculated using FID and response factors obtained from calibration curves constructed with the data obtained for individual thioesters (**C3**, **C4**, **C6**, **C10** and **C14**) or estimated (*) via linear regression of data for the pure compounds.

In conclusion, the feasibility of a "one pot" synthesis of flavor libraries has been established by reacting methyl chlorothiolformate with 11 carboxylic acids. The resulting library was found to be very clean and contained one major impurity, *S*,*S*-dimethyldithiocarbonate, which was an expected side product of the reaction. The structure of each individual component of the library was confirmed by GC−MS and the molar

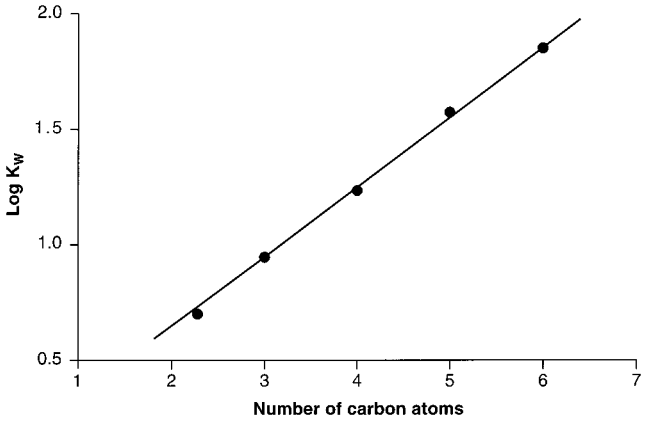


Figure 2. Relationship between log k_w and carbon chain length of *S*-methyl thioesters.

Table 3. Lipophilicity Coefficients (log k_w) of *S*-Methyl Thioesters and Relative Retention in Matrixes Containing Triolein

library component	log k_w	1/rel retention[c] (%)		
		10% triolein	20% triolein	33% triolein
C2	0.651[a]	58	42	36
C3	0.936[a]	50	34	24
C4	1.239[a]	40	25	14
C5	1.578[a]	26	15	7
C6	1.840[a]	18	9	4
C8	2.457[b]	5	3	1
C10	3.061[b]	1	<1	<1
C12	3.665[b]	<1	<1	<1
C14	4.269[b]	<1	<1	<1
C16	4.873[b]	<1	<1	<1
C18	5.477[b]	<1	<1	<1

[a] The values obtained were determined experimentally using reverse-phase HPLC. [b] These values were calculated by extrapolation of log k_w values obtained from **C2**−**C6** (see Figure 2). [c] The relative retention of thioesters by triolein was calculated from the following equations: $\text{retention}_{10\%\ \text{triolein}} = 0.0611k_w + 1.4587$, $\text{retention}_{20\%\ \text{triolein}} = 0.1306k_w + 1.7758$, and $\text{retention}_{33\%\text{triolein}} = 0.3258k_w + 1.3260$.

concentrations of *S*-methyl thioesters were determined by two independent methods using GC equipped with SCD and FID. It was found that with the exception of *S*-methyl thioacetate (**C2**, 0.8 mol %), molar concentrations of the other *S*-methyl thioester homologues varied in a relatively narrow range from 4.2 mol % for *S*-methyl thiopropionate (**C3**) to 14.1 mol % for *S*-methyl thiohexanoate (**C6**) with the maximum value obtained for medium chain thioesters. This variation was attributed to the partial loss of the most volatile components during extraction and the slightly lower chemical reactivity of longer chain carboxylic acids used during the synthesis of the thioesters. The library was also used to determine lipophilicity coefficients (log k_w) and the retention of *S*-methyl thioesters by 10, 20, and 33% triolein to illustrate that these parameters can be obtained for a relatively large number of compounds in a single experiment. In addition, as described in the accompanying paper (Berger et al., 1999) the library can also be used for simultaneous sensory analysis as well as for the identification of new flavor compounds.

ACKNOWLEDGMENT

The authors are grateful to Sylvie Breton, Antoine Follin, Michèle Dame-Cahagne (SOREDAB S.A., France), Pascal Molimard, and Michel Brousse (SKW Biosystems, France) for most fruitful discussions and suggestions. We also wish to thank Claudia Kuhn (sponsored by Christian Hansen A/S, Denmark) for performing experiments at a later stage in this investigation.

LITERATURE CITED

Berger, C.; Martin, N.; Collin, S.; Gijs, L.; Khan, J. A.; Piraprez, G.; Spinnler, H. E.; Vulfson, E. N. Combinatorial approach to flavor analysis. 2. Olfactory investigation of a library of *S*-methyl thioesters and sensory evaluation of selected components. *J. Agric. Food Chem*. **1999**, *47*, 3274−3279.

Bosset, J. O.; Gauch, R. Comparison of volatile flavour compounds of six European "AOC" cheeses by using a new dynamic headspace GC-MS method. *Int. Dairy J*. **1993**, *3*, 359−377.

Chaiken, I. M., Janda, K. D., Eds.; *Molecular diversity and combinatorial chemistry*; American Chemical Society: Washington, DC, 1996.

Cuer, A.; Dauphin, G.; Kergomard, A.; Roger, S.; Dumont, J. P.; Adda, J. Flavour properties of some sulfur compounds isolated from cheeses. *Lebensm.-Wiss. Technol.* **1979**, *12*, 258−261.

Dumont, J. P.; Roger, S.; Adda, J.; Etude des composés volatiles neutres presents dans les fromages à pâte molle et croûte lavée. *Lait* **1974**, *531*−*532*, 31−43.

Dumont, J. P.; Roger, S.; Adda, J.; L'arôme du Pont l'Evêque. Mise en évidence de constituants volatils quantitativement mineurs. *Lait* **1976**, *553*−*554*, 177−180.

El Tayar, N.; van de Waterbeemd, H.; Testa, B. Lipophilicity measurements of protonated basic compounds by reversed-phase high performance liquid chromatography. I. Relationship between capacity factors and the methanol concentration in methanol−water eluents. *J. Chromatogr*. **1985**, *320*, 293−304.

El Tayar, N.; van de Waterbeemd, H.; Testa, B. Lipophilicity measurements of protonated basic compounds by reversed-phase high performance liquid chromatography. II. Procedure for the determination of a lipidic index measured by reversed-phase high performance liquid chromatography. *J. Chromatogr*. **1985**, *320*, 305−312.

Gordon, E. M., Kerwin, J. F., Jr., Eds. *Combinatorial chemistry and molecular diversity in drug discovery*; Wiley: New York, 1998.

Kim, S.; Lee, J. I.; Kim, Y. C. A simple and mild esterification method for carboxylic acids using mixed carboxylic carbonic anhydrides. *J. Org. Chem*. **1985**, *50*, 560−565.

Obrecht, D., Villalgordo, J. M., Eds. *Solid-supported combinatorial and parallel synthesis of small-molecular-weight compound libraries*; Pergamon, Oxford, England, 1998.

Parliment, T. H.; Kolor, M. G.; Rizzo, D. J. Volatile components of Limburger cheese. *J. Agric. Food Chem*. **1982**, *30*, 1006−1008.

Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. Combinatorial synthesis − the design of compound libraries and their application to drug discovery. *Tetrahedron* **1995**, *30*, 8135−8173.

Wilson, S. R., Czarnik, A. W., Eds. *Combinatorial chemistry synthesis and applications*; Wiley: New York, 1997.

Received for review March 3, 1999. Revised manuscript received June 2, 1999. Accepted June 4, 1999. The authors are grateful to the EC Commission for the financial support of this work (GrantPL 96 1196).

JF9902043