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D I P L O M A R B E I T

Computer aided fragrance design- an update

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für meine Eltern

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Kutzfassung

Die Diplomarbeit "Computer aided fragrance design- an update" gliedert sich in zwei Teile. Im ersten Teil der Arbeit, auf Englisch geschrieben, wurden die neuesten Entwicklungen in der Riechstoffchemie, von Geruchsrezeptoren bis zu einzelnen Riechstoffen, die als Grundstoffe in der Parfüm-Produktion verwendet werden, wie Sandelholz, Ambra, Moschus, beschrieben. Als Quelle wurden wissenschaftliche Arbeiten, mit dem Schwerpunkt auf neuesten Studien seit 2004, verwertet.

Im zweiten Teil, in deutscher Sprache verfaßt, wurde im Rahmen eines Projekts des Wiener Wissenschafts-, Forschungs- und Technologiefonds (WWTF) – "*Haptic and Olfactory Design, Resources for Vienna's Creative Industrie",* der Geruch, d.h. die Duftstoffe, die in einem Antiquariat vorkommen, untersucht. Die Proben wurden mittels solid phase microextraction, SPME-Methode eingesammelt und dann mit Hilfe der Gaschromatographie-Massenspektrometrie, GC-MS Technik getrennt und analysiert. Die im Chromatogramm getrennten Peaks wurden einzeln ausgewertet und mit Hilfe von bekannten Datenbanken, bestehenden Arbeiten zum Thema ältere Bücher, sowie Publikationen auf dem Gebiet der Aroma-, Duft- und Riechstoffchemie, identifieziert. In den Chromatogrammen wurden flüchtige Abbauprodukte mit wichtigen Eigenschaften für die Erhaltung von historischem Papier, von Harzen und Lignin wie Carbonylverbindungen, mittlere und höhere Aldehyde und Alkylcarbonsäuren, wie auch die Vielzahl von Materialien, die für Buch-Produktion verwendet werden (Papier, Tinte, Klebstoff...), gefunden.

INHALTSVERZEICHNIS:

ABBILDUNGSVERZEICHNIS:

I. Teil

1. Einleitung

Die olfaktorische Wahrnehmung (lat. *olfacere* ‚riechen'), auch Geruchssinn, der komplexeste chemische Sinn, bezeichnet die Wahrnehmung von Gerüchen.

Riechstoffe oder Geruchsstoffe umfassen alle natürlichen und synthetischen Stoffe, die in einem Geruch vorkommen. Dabei ist der Geruch eine Reaktion des Riechenden. Um als Geruch wahrgenommen zu werden, muss eine Substanz flüchtig, also gasförmig sein bzw. in den gasförmigen Zustand übergehen und darf eine bestimmte Molekülmasse nicht überschreiten. Flüssige oder feste Stoffe müssen also in ausreichendem Maße in den gasförmigen Zustand übergehen. Hierzu ist ein ausreichend hoher Dampfdruck des Stoffes erforderlich. Mit steigendem Molekulargewicht werden Stoffe schwerer flüchtig. Etwa ab einem Molekulargewicht von ca 300 reicht der Dampfdruck einer Substanz nicht mehr aus, um die zur Erzeugung eines Geruchsreizes notwendige Konzentration aufzubringen.

Am Geruchssinn sind zwei sensorische Systeme beteiligt: das olfaktorische und das trigeminale System. Geruch und Geschmack interagieren und beeinflussen einander gegenseitig. Geruchsrezeptoren (olfaktorische Rezeptoren) sind auf chemische Reize reagierende Rezeptoren, die insbesondere an der Wahrnehmung des Geruchs beteiligt sind. Bei Geruchsrezeptoren handelt es sich um G-Protein-gekoppelte Rezeptoren. Da die Geruchsrezeptoren chiral sind, rufen Enantiomere eines Riechsstoffes einen unterschiedlichen Geruch hervor.

Im ersten Teil der Arbeit wurden die neuesten Entwicklungen in der Riechstoffchemie, von Geruchsrezeptoren bis zu einzelnen Riechstoffen, die als Grundstoffe in der Parfüm-Produktion verwendet werden, wie Sandelholz, Ambra, Moschus, beschrieben. Als Quelle wurden wissenschaftliche Arbeiten, mit dem Schwerpunkt auf neuesten Studien seit 2004, verwertet.

Einer der größten Erfolge bei der Erforschung des Geruchssinns gelang den beiden amerikanische Forschern Linda B. Buck und Richard Axel, die mit ihren Genforschungen etwa 1000 für die Geruchsrezeptoren verantwortlichen Gene identifizieren konnten und dafür 2004 den Nobelpreis für Medizin erhielten.

2. Odor and the Sense of Smell- A review

An odor is caused by one or more volatilized chemical compounds, generally at a very low concentration, that humans or other animals perceive by sense of olfaction. Olfaction registers chemical information in organisms ranging from insects to humans, including marine organisms. The typical stimulus is an organic chemical with molecular weight below 300 daltons. A few inorganic chemicals, such as ammonia, halogens, hydrogen sulfide, can also stimulate olfactory receptors and the *Nervus Trigeminus*.

The anatomy of olfactory structures and the neurophysiology of olfaction differs significantly among different animal groups. For example, insect olfactory receptors exist within sensory hairs on the antennae. The olfactory organ of fishes resides in tubular chambers of the mouth. In terrestrial vertebrates, the olfactory receptors reside within a sac or cavity more or less similar to the human nasal cavity. The olfactory mucosa patch in the cavity contains millions of receptor cells, though in some olfactory-dominated mammals, such as the dog and rabbit, it contains tens of millions [1].

In human the mucosa is situated away from the main airstream. During quiet breathing eddy currents may carry just enough stimulus to evoke a sensation, whereupon sniffing will occur. Sniffing amplifies the amount of stimulus reaching the receptors by as much as tenfold.

The stimuli for olfaction are commonly complex, they are mixtures. Such products as perfumes contain at least hundreds of odor-relevant constituents. Only rarely does the distinctive quality of a natural product arise from only one single constituent. A chemical analysis of most products will not usually allow a simple prediction of odor intensity or quality. One general rule is that the perceived intensity of the mixture falls well below the sum of the intensities of the unmixed components [2].

Human olfactory sensitivity varies from odorant to odorant over several orders of magnitude. A common range of thresholds for materials used in fragrances is 1 to 100 parts per 10^9 parts of air. Thresholds gathered from various groups of human subjects permit certain generalities about how the state of the organism affects olfaction. For instance, persons aged 50 and above are about tenfold less sensitive than young adults.

An organism must not only be living to experience the sense but also must have a functioning nervous system. Persons with certain medical disorders, such as multiple sclerosis, Parkinson's disease and olfactory tumors, exhibit decreased sensitivity (hyposmia) or complete absence of sensitivity (anosmia) [1].

The properties that endow a molecule with its quality have spawned more theories of olfaction. Most modern theories hold that the key to quality lies in the size and shape of molecules, with some influence of chemical functionality. For molecules below about 100 daltons, functional group has obvious importance: for example, esters smell fruity, amines fishy-uriny and carboxylic acids rancid. For larger molecules, the size and shape of the molecule seem more important. Shape detection is subtle enough to enable easy discrimination of some optical isomers. Progressive changes in molecular architecture along one or another dimension often lead to large changes in odor quality [1].

The difficult task of our nose to detect and discriminate among thousands of low-molecular-weight organic compounds with diverse chemical structures and properties requires an enormous molecular recognition capacity. This is based on distinct proteins, capable of recognizing and binding odorous compounds, including odorant-binding proteins, which are supposed to shuttle odorous compounds through the nasal mucus, and most notably the odorant receptors, which are heptahelical membrane proteins coupling via G-proteins onto intracellular transduction cascades. From more than a thousand genes each olfactory neuron is supposed to express only one receptor subtype [3].

3. Olfactory system

Smelling is a direct experience, because we inhale microscopic portions of substances that have evaporated and make their way into the nasal cavity, where they chemically interact with sense receptors. Cells in the nose detect odors through receptor proteins on the cell surface, which bind to odor-carrying molecules. A specific odorant docks with an olfactory receptor protein, the way that a key fits into a lock and this excites the nerve cell, causing it to send a signal to the brain.

The olfactory cells of vertebrates, usually located in the olfactory mucosa of the upper nasal passages, are specialized neutral elements that are responsive to chemicals in the vapor phase. The limbic system of the brain, which modulates appetitive and emotional behavior and hedonic experiences provides the neural substrate for the pleasure or displeasure of sensations.

To be experienced through the senses, all data must be transmitted to the brain through the nervous system. This happens in receptors through the conversion and transmission of physical or chemical information. A receptor is a structure in the nervous system that receives specific stimuli and is affected in such a way that it sends particular messages to the brain. The brain interprets these messages as sensations corresponding to the stimuli [1].

3.1. Mechanism of the Olfactory system

The mechanism of the olfactory system can be divided into a peripheral one, sensing an external stimulus and encoding it as an electric signal in neurons, and a central one, where all signals are integrated and processed in the central nervous system [2].
In mammals, the main olfactory system detects odorants that are inhaled through the nose, where they contact the main olfactory epithelium, which contains various olfactory receptors. These olfactory receptors are membrane proteins of bipolar olfactory receptor neurons in the olfactory epithelium. Rather than binding specific ligands like most receptors, olfactory receptors display affinity for a range of odor molecules. Olfactory neurons transduce receptor activation into electrical signals in neurons. The signals travel along the olfactory nerve, which belongs to the peripheral nervous system. This nerve terminates in the olfactory bulb, which belongs to the central nervous system. The complex set of olfactory receptors on different olfactory neurons can distinguish a new odor from the background environmental odors and determine the concentration of the odor [1].
The central neural pathways of the olfactory system have a complexity unmatched among the sensory systems. One pathway carries information to the pyriform cortex, the hypothalamus, and other structures of the limbic system. The strong affective and motivational consequences of olfactory stimulation seem compatible with projections to the limbic system and with the role of olfaction in certain types of physiological regulation. In many vertebrate species, reception of pheromones occurs via an important accessory olfactory organ, known as the vomeronasal organ, which characteristically resides in the hard palate of the mouth or floor of the nasal cavity. The piriform cortex is the area most closely associated with identifying the odor. The medial amygdala is involved in social functions such as recognition of animals of the same species. The entorhinal cortex is associated with memory, e.g. to pair odors with proper memories [1].

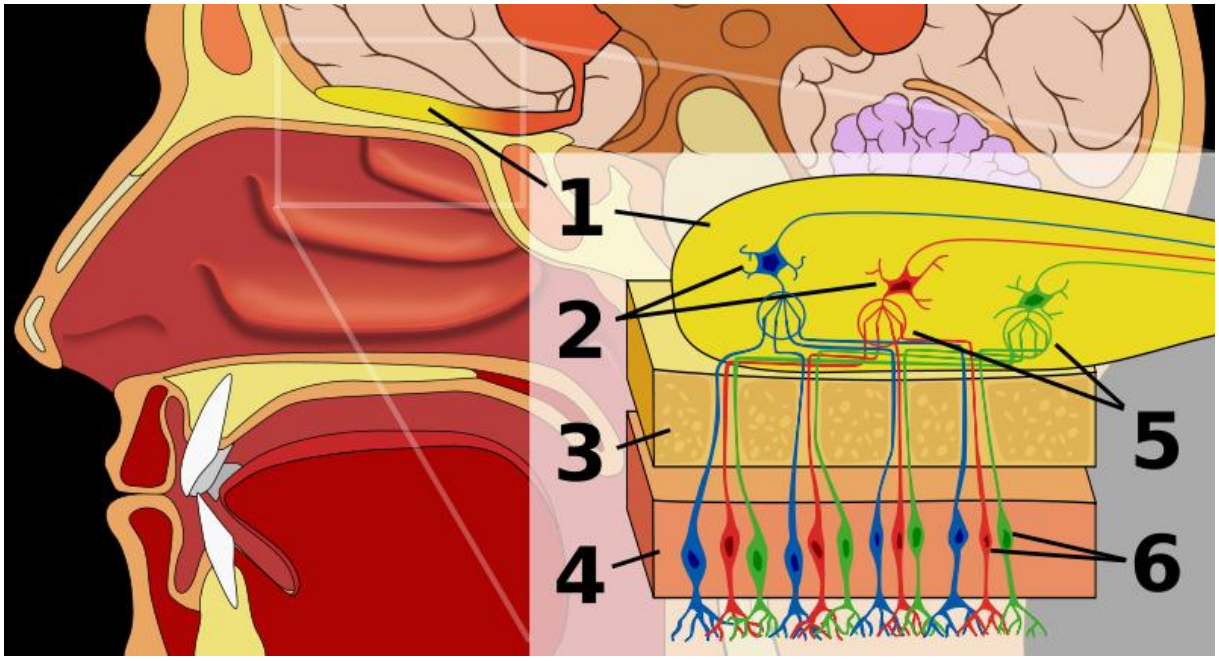

Abbildung 1 Olfactory receptor cells

1: Olfactory bulb 2: Mitral cells 3: Bone 4: Nasal epithelium 5: Glomerulus 6:

(from Image:Brain human sagittal section by Lynch [4])

Reception of the chemical stimulus and transduction into a neural signal apparently occur on the olfactory receptor cilia. The ciliary membrane contains receptor protein molecules that interact with stimulating molecules through reversible binding. Adjacent points in the mucosa generally project to adjacent points in the olfactory bulb of the brain. The synapses between the incoming olfactory nerve fibers and the second-order cells, mitral cells, occur in basketlike structures called glomeruli. On average, a glomerulus receives about 1000 receptor cell fibers for each mitral cell. The location of cells within the bulb seems to play a role in encoding odor quality: each odorant stimulates a more or less unique spatial array [1].

3.2 Olfactory receptors (ORs)

Buck and Axel have discoverd that in mammals there are ~1000 different ORs, which belong to the large gene family of G protein-coupled receptors [5].

ORs expressed in the cell membranes of olfactory receptor neurons are responsible for the detection of odor molecules. Activated ORs are the initial player in a signal transduction cascade which produces a nerve impulse which is transmitted to the brain. These receptors are members of the class A rhodopsin-like family of G protein-coupled receptors. ORs display affinity for a range of odor molecules, and conversely a single odorant molecule may bind to a number of ORs with varying affinities. Once the odorant has bound to the OR, the receptor undergoes structural changes and it binds and activates the olfactory-type G protein on the inside of the OR-neuron. The G protein (G_{olf} and/or G_s) in turn activates the lyase - adenylate cyclase - which converts ATP into cyclic AMP (cAMP). The cAMP opens cyclic nucleotide-gated ion channels which allow calcium and sodium ions to enter into the cell, depolarizing the OR neuron and beginning an action potential which carries the information to the brain [6].

There are a large number of different ORs, with as many as 1000 in the mammalian genome which represents approximately 3% of the genes in the genome. However not all of these potential OR genes are expressed and are functional. According to an analysis of data derived from the human genome project, humans have approximately 400 functional genes coding for ORs and the remaining 600 candidates are pseudogenes [7].

The names of individual OR family members are in the format "ORnXm", for example *OR1A1* is the first isoform of subfamily A of OR family 1.

Members belonging to the same subfamily of OR (>60% sequence identity) are likely to recognize structurally similar odorant molecules [8].

Two major classes of ORs have been identified in humans [9]:

class I (fish-like receptors) OR families 51-56

class II (tetrapod specific receptors) OR families 1-13.

The relationship between molecular structure and odor character is one of the most complex structure–activity problems in biology. Despite over a century of effort, it remains unsolved, and synthesis of new odorants still proceeds largely by trial and

error. Turin [10] have argued that the reason for this failure lies in a mistaken assumption, that molecular shape determines odor character. In this study, using semi-empirical quantum chemistry methods and a simple calculation method for tunnelling mode intensities, Turin calculated the spectra of structurally diverse odorants belonging to various odor categories. With few exceptions, the calculated spectra of bitter almonds, musks, ambers, woods and sandalwoods strongly correlate with odor character. Despite its simplicity, the method for spectrum calculation described here is strikingly successful at predicting similarities and differences in odor character between odorants belonging to widely different structural and odor classes [10].

Computerized approach to structure-odor relationships (SOR), has allowed many new discoverys on this field. In olfactory neurons, expression of a single odorant receptor OR from a repertoire of >1,000 genes is required for odor coding and axonal targeting.

The daunting task of our nose to detect and discriminate among thousands of low-molecular-weight organic compounds requires an enormous molecular recognition capacity. This is based on distinct proteins, capable of recognizing and binding odorous compounds, including odorant-binding proteins, which are supposed to shuttle odorous compounds through the nasal mucus, and most notably the odorant receptors, which are heptahelical membrane proteins coupling via G-proteins onto intracellular transduction cascades. From more than a thousand genes each olfactory neuron is supposed to express only one receptor subtype. Receptors appear to be selective but rather non-specific – i.e. a distinct odorant activates multiple receptors and individual receptors respond to multiple odorants [3].

The search to correlate the molecular structure and the odor character of a chemical compound has a long recorded history. The development of synthetic organic chemistry in the 19th century, have alowed chemists to understand the relationship between molecular structure and the odor of a molecule, largely with the intention to design novel molecules with desirable odor properties.

Most structure–odor models are concerned with the character of the odor. However, the commercially important parameters of detection threshold, recognition threshold, and superthreshold intensity of odorants have received much less attention.

Detection threshold models are beginning to appear and examples include studies by Kraft on materials with marine and musk odor characters [11].

The discovery of the odorant receptor family by Buck and Axel in 1991 provided a quantum jump in our understanding of olfactory function [5].

Studies from Lewcook and Reed [12] demonstrate the importance of OR protein in the establishment of selective OR expression. This protein therefore participates in three diverse and complex aspects of olfactory function: odorant recognition, axon pathfinding, and the regulation of receptor expression.

They have demonstrated a role for OR protein as an essential regulator in the establishment of monoallelic OR expression. An OR-promoter-driven reporter expresses in a receptor-like pattern but, unlike a native OR, is coexpressed with an additional OR allele. The presence of an untranslatable OR coding sequence in the mRNA is insufficient to exclude expression of a second OR. These data identify the OR protein as a critical element in a feedback pathway that regulates OR selection [12].

The use of kinetic resolution mediated by lipases is a flexible and widely applicable method for the preparation of the stereoisomers of chiral fragrant substances in enantiomerically enriched or enantiomerically pure form. This method requires only the identification of a suitable alcohol or acetate as substrate for the biocatalyzed transesterification or hydrolysis. After resolution, simple and straightforward chemistry can usually be employed to convert the intermediates into the final odorants in good yields. The enzyme-mediated approach allowed us rapidly to obtain samples of high enantiomeric purity for olfactory evaluations [13].

Enantiomers, chiral pairs of left- and right-handed structures, are an important class of molecules in proposed mechanisms. Brookes et al.[14] showed that there is a correlation between molecular (structural) flexibility and whether or not the left- and right-handed enantiomers smell the same. In particular, for the fairly extensive class of enantiomers with six-membered ring flexibility, enantiomers do not smell the same. The differences in scent of these enantiomers appear to be consistent with simple generalizations of a 'swipe card' model in which, while the shape must be good enough, critical information for activation is a separate factor [14].

The paper from Tromelin et al. [15] describes 2D- and 3D-QSAR models of interaction between flavor compounds and β-lactoglobulin, using an application of

Catalyst to describe three aroma sets to generate activities-based alignments, using the best generated hypotheses. The obtained Catalyst models confirmed the existence of at least two binding sites on the β-lactoglobuline [15].

Further research by Guth et al. [16] has shown that binding affinitives for flavor compounds on various biopolymers can be estimated by calculation of physico-chemical descriptors for the odorants. A β-lactoglobulin-lacton binding position has been identified and confirmed by competitive binding studies. A model has been developed to estimate the free energy of binding of odorants to biopolymers. Binding affinitives of δ- and γ-lactones to bovine serum albumin were investigated by ultracentrifugation and equilibrium-dialysis techniques. QSAR of lactone binding on proteins were perfomed by the measurement of lipophilicity and H-bond strength. Large differences in observed protein-binding properties for the various compounds demonstrated that structure-activity relationship was significantly influenced by the lipophilicity by the odorant. If the structure of the receptor molecule is known, computational ligand-macromolecule docking experiments can be used to predict binding affinities for unknown compounds with receptor molecul [16].

As in the previous studies of binding lactones and odorant interaction, computer-aided modeling was also used in aroma design in an article from Korichia et al. [17] Aroma molecules are found in a wide variety of products ranging from perfumes, health care products and medicines. In this paper, the methodology for computer aided aroma design was presented.

Computer aided aroma design (CAAD) is likely to become a hot issue as the REACH-EC document targets many aroma compounds to require substitution. The two crucial steps in computer aided molecular design (CAMD) are the generation of candidate molecules and the estimation of properties, which can be difficult when complex molecular structures such as odors are sought. The CAAD-methodology is based on the multi-level framework of the CAAD-methodology but with extensions. The multi-level of the molecular screening approach is formally matched by a molecular framework, that uses molecular graph concepts and routines to be able at each level to provide information for evaluation using property estimation methods which complexity increases as one moves up one level.

It can distinguish the infinitesimal chemical structure differences, such as in isomers, that are responsible for different odor quality and intensity [17].

The quantitative-structure odor approach were used in next studies.

In the next study a group of scientists have evaluated the statistical link between OR1G1 response to odorants, 3D-QSAR categorization of OR1G1 ligands, and their olfactory description. They demonstrated that OR1G1 recognizes a group of odorants that share both 3D structural and perceptual qualities.

Recent studies (Schmiedeberg et al. [18]; Stary et al. [20]) demonstrated that ORs with high homology (orthologues such as human OR1A1 and mouse Olfr43 or paralogues such as human OR1A1 and human OR1A2) bind common ligands with similar efficacy, whereas ORs more distantly related (such as Olfr43 and Olfr49) binds common odorants but with different efficacies. The study by Schmiedeberg [18] also demonstrated that evolutionary conserved amino acid positions define the ligand-binding site. Then, these studies suggest that an odorant would be recognized via a similar odotope by closely related ORs, whereas distantly related ORs would bind a common ligand via different odotopes.

In the present work, scientists have demonstrated that an OR can recognize 2 odotopes, suggesting that the binding pocket of an OR can accommodate several odotopes. When taken together, their results suggest that odorants sharing a same odotope recognized by OR1G1 would evoke similar odor quality. The findings reported here provide a new insight in the understanding of the relationships between odorants, ORs and odor quality.

It has especially been shown by a 3D-QSAR approach that ligands of an OR, OR1G1, have to be divided in 2 groups in order to find satisfactory models, suggesting 2 modes of interaction of odorants with this receptor. This result is in agreement with another study by Sell [11] reporting that it would be difficult to design a model for a typical ligand for OR1G1.

In another part of this work, it was also the likely involvement of OR1G1 in the perception of waxy, fatty, and rose odor in humans reported. These results support the idea that, among the specific group of ORs activated by an odorant and defining its particular odor perception, some ORs that strongly bind this odorant might determine its major odor quality [19, 20].

This chapter by Lavine et al. [21] describes a new odor structure relationship (OSR) using electronic van der Waals surface property descriptors and genetic algorithms, which have proved very successful in comparison to previous methodes. OSR

correlation methodology utilizes large olfactory databases available in the open scientific literature as input. The first step in this procedure is to represent each molecule in the database by an appropriate set of molecular descriptors. Breneman's Transferable Atom Equivalent (TAE) descriptor methodology is used to create a large set of electron density derived shape/property hybrid descriptors. These descriptors have been chosen because they correlate with key modes of intermolecular interactions and contain pertinent information about shape and electronic properties of molecules. In contrast to more traditional methodologies that have shown not to be effective, the use of shape-aware electron density based molecular property descriptors has eliminated many of the problems associated with the use of descriptors based on substructural fragments or chemical topology. A second reason for the limited success of past OSR efforts can be traced to the complex nature of the underlying modeling problem. There has been developed a genetic algorithm for pattern recognition analysis that selects descriptors, which create class separation in a plot of the two or three largest principal components of the data. Because principal components maximize variance, the bulk of the information encoded by these descriptors is about differences between the odorant classes in a data set [21].

Quantitative structure–activity relationship models were successfully developed by Du et al.[22] for predicting the relative sensitivities odor detection thresholds and nasal pungency thresholds for the olfaction and nasal trigeminal chemosensory systems of a set of volatile organic compounds. The best multi-linear regression method was used to select the most important molecular descriptors and build a linear regression model. The methods support vector machine and local lazy regression (LLR) were also used to build regression models. By comparing the results of these methods for the test set of odor detection thresholds (ODTs) and nasal pungency thresholds (NPTs), the LLR model gave better results for the VOCs with the coefficient of determination R^2 (0.9171, 0.9609, respectively) and root mean square error (0.3861, 0.2152, respectively). At the same time, this study identified some important structural information which was strongly correlated to the relative sensitivities of these VOCs. As it could predict accurately the relative sensitivities of the olfaction and nasal chemesthesis, the LLR method is a promising approach for QSAR modeling [22].

Relying on a previous study Abraham et al.[23] have applied a quantitative structure–activity relationship (QSAR) approach to analyze the chemical parameters that determine the relative sensitivity of olfaction and nasal chemesthesis to a common set of volatile organic compounds (VOCs). Previously reported data on odor detection thresholds (ODTs) and nasal pungency thresholds (NPTs) from 64 VOCs belonging to 7 chemical series (acetate esters, carboxylic acids, alcohols, aliphatic aldehydes, alkylbenzenes, ketones, and terpenes) were used. The analysis tested whether NPTs could be used to separate out "selective" chemosensory effects from "specific" chemosensory effects in ODTs. Previous work showed that selective effects dominate chemesthetic potency whereas both selective and specific effects control olfactory potency. Authors have concluded that it is indeed possible to use NPTs to separate out selective from specific effects in ODTs. Among the series studied, aldehydes and acids, except for formic acid, show clear specific effects in their olfactory potency. Furthermore, for VOCs whose odor potency rests mainly on selective effects, these have been developed a QSAR equation that can predict their ODTs based on their NPTs [23].

The key finding of this manuscript "Predicting Odor Pleasantness from Odorant Structure: Pleasantness as a Reflection of the Physical World" by Khan et al.[24] was that 144 molecules were similarly ordered by two independently obtained principal axes, one for perception and one for physicochemical structure.

It was shown that when physicochemical measuremants with no *a priori* connection to any particular percepts were analyzed, those physicochemical measurements that were best at discriminating a set of molecules were found to be those that were most correlated with the perception of olfactory pleasantness. In other words, when one orders a set of odorants based on the variance in their physicochemical properties alone, they end up roughly ordered by perceptual pleasantness as well. This phenomenon allowed to predict odorant pleasantness of 50 molecules that authors did not smell previously, that were here tested in 80 subjects spanning three cultures. This ability to predict perceptual properties of novel odorants was a critical aspect of this manuscript [24].

Hummel [25] reported about developement of stimulation techniques that allow controlled ortho- or retronasal presentation of odorous stimuli. Results from psychophysical, electrophysiological and imaginig studies suggest that there are

clear differences in the perception of ortho- and retronasal stimuli. The reason can be found in hypothesizing that the sorption of odors to the olfactory epithelium in relation to the direction of airflow changes the pattern of mucosal activation and consequently the perception of the same odor in relation to the route of presentation [25].

It has long been believed that vertebrate olfactory signal transduction is mediated by independent multiple pathways (using cAMP and Inositol 1,4,5-triphosphate ($InsP_3$) as second messengers). However, the dual presence of parallel pathways in the olfactory receptor cell is still controversial. In this study, activities of transduction channels of single olfactory receptor cells to $InsP_3$-producing odorants have been recorded. Actually, $InsP_3$-producing odorants generated responses in a smaller fraction of cells (lilial, 3.4%) than the cAMP-producing odorant (cineole, 26%). By applying both types of odorants alternatively to the same cell, furthermore, Takeuchi et al. [26] have observed cells to exhibit symmetrical cross-adaptation. It seems likely that even with odorants with different modalities adaptation occurs completely depending on the amount of current flow. The data have also showed that olfactory response generation and adaptation are regulated by a uniform mechanism for a wide variety of odorants [26].

Industrial and agricultural off-gas streams are comprised of numerous volatile compounds, many of which have substantially different odorous properties. The aim of this paper from Mahlke et al. [27] is to identify possible model substances in selective odor separation research from 155 volatile molecules mainly originating from livestock facilities, fat refineries, cocoa and coffee production by knowledge-based methods. All compounds are examined with regard to their structure and information-content using topological and information−theoretical indices. Resulting data are fitted in an observation matrix, and similarities between the substances are computed. Principal component analysis and k-means cluster analysis are conducted showing that clustering of indices data can depict odor information correlating well to molecular composition and molecular shape. Quantitative molecule description along with the application of such statistical means therefore provide a good classification tool of malodorant structure properties with no thermodynamic data needed. The approximate look-alike shape of odorous compounds within the clusters suggests a fair choice of possible model molecules [27].

The human nose detects volatile chemical stimuli by at least three different receptor families: odorant receptors, trace amine-associated receptors (TAARs), and vomeronasal type-1 receptors (VN1Rs). All members of the three odorant receptor families belong to class A (rhodopsin-like) G protein-coupling receptors (GPCRs).

However, little is known about specific differences in the functional designation of the three olfactory receptor families, subjects dealt with by Krautwurst [28] in this work. Human ORs detect odorants in a combinatorial way, such that each receptor recognizes several odorants, and one odorant activates several ORs. Some members of the other two human olfactory receptor families, TAARs and VN1Rs, have also been shown to be specifically activated by several volatiles of certain chemical groups and in a combinatorial way [28].

Calcium-activated chloride channels (CaCCs) are involved in many physiological processes, including sensory signal transduction, but only little is known about their structure and function. In new studies from Rasche et al. [29] a proteome analysis of the olfactory epithelium (OE) membrane proteome has been performed and identified so far uncharacterized membrane proteins as candidate channels. One of the most abundant membrane proteins in olfactory sensory neurons (OSNs) was Tmem16b, a member of a recently identified family of CaCCs. In addition to former studies performed on Tmem16b, here was showed that Tmem16b expression is highly specific for the OE, in contrast to the closely related Tmem16a, which shows a broad expression pattern in secretory epithelial cells. Native Tmem16b is localized in the cilia of the OSNs, which is in agreement with previous electrophysiological recordings [29].

Lai et al. [30] have simulated an odor ligand's dynamic behavior in the binding region of an olfactory receptor (OR). Their work has been carried out using the first published computational model of rat I7, the first identified OR (Singer [31]). Barring the availability of an OR crystal structure, the only structurally comparable aspects of all GPCRs are helical TM domains.

Their results have shown that for a ligand to activate a receptor, it should be dynamically stable in the receptor-binding region. Steric factors play an important role in such stabilizations. In most of the simulations, even if the ligand is not docked in a position for a facile exit, when conformational changes allow it (after as

long as 100 ps) to be in the exit pathway, an exit occurs, that shows that such a path in and out of the binding pocket exists.

The short timescale computational studies (up to 200 ps) have helped to identify unprecedented postdocking ligand behavior of ligands. From *in vacuo* molecular dynamics simulations of interactions between models of rat OR I7 and 10 aldehyde ligands, they have identified a dissociative pathway along which the ligand exits and enters the OR-binding pocket a transit event. The ligand's transit through the receptor's binding region may mark the beginning of a signal transduction cascade leading to odor recognition. Results have helped to substantiate or to refuse previously held notions of amino acid contribution to ligand stability in the binding pocket.

Their observations of ligand activity when compared to those of experimental (electroolfactogram response) OR-activation studies provide a view to predicting the stability of ligands in the binding pocket as a precursor to OR activation by the ligand [30].

A recent structural bioinformatic analysis suggests that structural features are conserved across the class of GPCRs in spite of their low sequence identity. Based on this work, Khafizov et al. [32] have aligned the sequences of 29 ORs for which ligand binding data are available. Findings in this work are consistent with most of the previous models and allow predictions for site-directed mutagenesis experiments. Modeling provides a rationale for amino acids in equivalent positions in most of the odorant receptors considered and helps to identify other amino acids that could be important for ligand binding. In the case of mutagenesis receptor MOR42-1 and MOR42-3, which bind dicarboxylic acids, scientists have proposed the presence in the binding pocket of two polar regions, constituted by several residues [32].

Receptor-ligand interaction models are generally based on a 'lock and key' concept. In this studies, group of Triller et al. [33] have investigated the response of a human olfactory receptor, OR1D2, to a broad array of odorants and found that there is no direct correlation between a molecule's ability to activate this receptor and the odor impression elicited in the brain. In a parallel study on specific anosmia, they have found no evidence for odor-specific anosmia to either musk or amber. Their results show that simplistic 'lock and key' models of olfaction based on a concept of odor-quality-tuned receptors are inadequate, irrespective of the nature of the lock-key

interaction. Receptor activation is only one step in a long chain of events leading from inhalation of odorants to perception of odor in the higher brain, and, therefore, although structure-odor correlations are useful tools for the design of novel odorants, caution should be exercised when extrapolating them to models of olfactory perception [33].

In prediction of perception by probing the hOR17-4, in study from Doszczak et al. [34], silicon analogues of the lily-of-the-valley odorants lilial and bourgeonal were used to demonstrate that the electronic surface structure determines the interaction of an odorant with its olfactory receptor. The subtle changes in the stereoelectronic properties enable a comparison of *in vivo*, *in vitro*, and *in silico* data. Odor thresholds correlate well with the binding energies obtained from a computational model of the hOR17-4 receptor [34].

Next paper presents a prediction method, combining fuzzy logic and genetic algorithms, of the camphor odor. This method allows the chemist to localize the eventual optimal parameters responsible for a given odor or an activity in general. Kissi et al. [35] have used the Fuzzy C-Means Clustering (FCM) method to predict the class of all 99 molecules using the Zadeh, Lukasiewicz and the Ordered weighted averaging (OWA) operators. For each molecule, the method generates two degrees of odor response to be determined within 0 to 1. The rules used to discriminate between camphor and non camphor molecules lead to 77% correct discrimination. Such rules account for the shape and the size of the molecule. Their adjustment by means of genetic algorithms led to 84% correct discrimination between camphor and non-camphor molecules [35].

A fuzzy logic was used in comparing the information content of two large olfactory databases. A representative example is obtained by comparing the odorous compounds included in the "Perfumery Materials and Performance 2001" (PMP2001) database. A systematic analysis allows the isolation of about 900 shared molecules, and amongst them, only 2% recover the same olfactory descriptors, whereas 40% of them have a totally different profile, in which no odor included in a description can be found in the other one.

The objective of this paper consisted in defining a criterion able to compare the information content of two or more databases. This was achieved by using a data mining procedure based on the AFP method. AFP is a supervised classification

method implementing a fuzzy partition algorithm and it was already fully presented and validated before. It models relations between molecular descriptors and activities by dynamically dividing the descriptor space into a set of fuzzy partitioned subspaces defined by fuzzy rules. The aim of the algorithm is then to select the descriptor and the cut position, which allow retrieval of the maximal difference between the two fuzzy rule scores generated by the new subspaces. The score is determined by the weighted average of the activity values in an active subspace and in its neighboring subspaces. These models allowed the definition of four descriptor odor relationships, one for each olfactory note, and the parameters used for developing them were tuned with help of the validation set. The robustness and the prediction power of these models give a powerful criterion for evaluating the "quality" of their information content and for deciding which is the most trustable database [36].

3.3 Mammalian Olfactory system

The **mammalian olfactory system** is not uniformly organized but consists of several subsystems each of which probably serves distinct functions. Not only are the two major nasal chemosensory systems, the vomeronasal organ and the main olfactory epithelium, structurally and functionally separate entities, but the latter is further subcompartimentalized into overlapping expression zones and projection-related subzones.

Animals constantly survey their external environment for chemicals advising food sources and habitats but also for signals controlling social interaction and reproductive behavior. The chemical compounds are sensed by monitoring the respiratory airstream through the chemosensory neurons, which are organized in structurally and functionally divergent subsystems in the nasal cavity. Generally, two systems are distinguished: the main olfactory epithelium (MOE), which is considered to be responsible for sensing and discriminating the myriads of volatile odorous compounds, and the vomeronasal organ (VNO), which is thought to mediate the detection of substances carrying specific information concerning species, gender and identity of an animal.

However, taking into account that the rat OR repertoire is 2–3 times larger than the human one, there is a high chance that rats can smell the compounds and that at least some ORs respond to sandalwood olfactophores [9].

Liu et al. [37] have used an automatic and unsupervised system to study the most updated mammalian OR family of more than 1300 member genes and to a nearly complete database of mammalian odor receptor genes. They have obtained a comprehensive list of potential functional regions or motifs and a corresponding taxonomy of classes such that members of each class are likely to share common functional properties. Extensive analysis of all the generated motifs indicated interesting regions that could be involved in specific functions and corresponding subgroups such that members of a subgroup could share the specific functions. The generated motifs and gene classification were subjected to extensive and systematic downstream analysis to obtain biological insights. Two major results from this

studies were: a map of sequence motifs that may correlate with function and the corresponding receptor classes in which members of each class are likely to share specific functions. However, none of these classifications correlate well with the limited functional data available for ORs [37].

This research was made in 2003, since then new discoveries were made such as a new class of OR, the TAARs. Liberles et al. [38] have reported the discovery of a second family of receptors in the mouse olfactory epithelium. Genes encoding these receptors, called 'trace amine-associated receptors' (TAARs), are present in human, mouse and fish. Like odorant receptors, individual mouse TAARs are expressed in unique subsets of neurons dispersed in the epithelium. These receptors are expressed in a small subpopulation of neurons that seem to lack odorant receptors, suggesting that these neurons use TAARs rather than odorant receptors to detect chemosensory stimuli. Similar to odorant receptors, different mouse TAARs are expressed in different neurons, and those with the same TAAR are scattered in selected olfactory epithelial regions. These studies show that at least four TAARs expressed in the mouse olfactory epithelium recognize small-molecule amines and, furthermore, that each of these receptors detects a unique set of amine ligands. These findings, together with the relatedness of TAARs to biogenic amine receptors, suggest that TAARs may specifically function as a family of chemosensory receptors for amines. Further, at least three mouse TAARs recognize volatile amines were found in urine: one detects a compound linked to stress, and the other two detect compounds enriched in male versus female urine [38].

Breer et al. [39] reported about multiple olfactory subsystems. For the two major nasal chemosensory systems, the MOE and the VNO, which are supposed to be involved in detecting common odorants and pheromones, respectively, this is reflected in different cell types (cilia vs. microvilli), different receptors and transduction cascades as well as projection sites into different brain regions.

Interspersed in the caudal recess of the nasal cavity are the so-called GC-D neurons, which express the receptor type guanylate cyclase-D and project axons to the necklace glomeruli. The populations of 'OR37' neurons express a unique class of highly conserved olfactory receptors and are also located in clustered manner as a distinct island at a particularly exposed site within the MOE. Although the adequate

odorous signals for this unique subsystem are still elusive, it is quite obvious that the mammalian-specific 'OR37' system plays a very special role in odor reception.

The most recently discovered subsystem, the so-called Grueneberg ganglion, is located in the rostral nasal vestibule far anterior of any of the other subsystems. It has been suggested that this subsystem may participate in receiving social signals most relevant during the early postnatal phase. Thus, the emerging picture indicates that the olfactory system comprises a variety of morphological, molecular and functional subsystems with defined projection patterns [39].

Floriano et al. [40] have used the MembStruk first principles computational technique to predict the three-dimensional (3-D) structure of six mouse olfactory receptors (S6, S18, S19, S25, S46 and S50) for which experimental odorant recognition profiles are available for a set of 24 odorants (carbons aliphatic alcohols, acids, bromo-acids and diacids). The HierDock method was used to scan each predicted OR structure for potential odorant binding site(s) and to calculate binding energies of each odorant in these binding sites. The calculated binding affinity profiles are in good agreement with experimental activation profiles, validating the predicted 3-D structures and the predicted binding sites. For each of the six ORs, the binding site is located between transmembrane domains (TMs) 3–6, with contributions from extracellular loops 2 and 3. In particular, scientists have found six residue positions in TM3 and TM6 to be consistently involved in the binding modes of the odorants. These predictions are also consistent with mutation data on ligand binding for catecholamine receptors and sequence hypervariability studies for ORs. Based on this analysis, they have defined amino acid patterns associated with the recognition of short aliphatic alcohols and mono-acids. Using these two sequence fingerprints to probe the alignment of 869 OR sequences from the mouse genome, 34 OR sequences were identified, matching the fingerprint for aliphatic mono-acids and 36 corresponding to the recognition pattern for aliphatic alcohols.

For the six mouse ORs studied here, the binding sites are located in the same region, between TM helices 3, 4, 5 and 6. This binding region contains a number of hypervariable residues among the ORs, consistent with their involvement in binding, as proposed in the literature (Malnic et al. [41]; Pilpel et al. [42]). Authors have speculated that these sets may also code odorants such as esters and aldehydes.

Two classes of ORs have been identified in vertebrates (Freitag et al. [43]): class I (fish-like receptors) and class II (mammalian-like receptors). Fish ORs respond to water-soluble odorants such as amino acids (Ivanova et al. [44]), while mammalian ORs respond to volatile compounds (Mezler et al. [45]). It has been suggested that class I ORs may be specialized in detection of water-soluble odorants, while class II detect volatiles (Freitag et al. [43]; Mezler et al. [45]). The two classes differ by the length of EC3 (longer in the fish-like class I ORs) and the sequence variability in TMs 3–5, although no obvious class-specific amino acid motif has been detected in the TM domains (Freitag et al. [43]; Mezler et al. [45]). Of the six ORs studied here, five (S18, S19, S46, S6 and S50) are selective for water-soluble odorants as expected for the fish-like class I ORs (Zhang et al. [46]), while S25 is selective for alcohols, consistent with class II. Since it was found that the preference of class I receptors for acids involves amino acid differences at positions TM3-6, TM3-9 and TM6-19 when compared with S25, one suggests that these positions might lead to class-specific fingerprints [40].

Phylogenetic analysis groups mammalian odorant receptors into two broad classes and numerous subfamilies. Abaffy et al. [47] have proposed these subfamilies to reflect functional organization, which variety of Class I and Class II mouse OR can be functionally expressed in Xenopus laevis oocytes. In this paper the receptive ranges of all members of the mouse odorant receptor 42 (MOR42) subfamily was examined. MOR42-1 responded to dicarboxylic acids, preferring a 10–12 carbon chain length. MOR42-2 responded to monocarboxylic acids (7–10 carbons). MOR42-3 responded to dicarboxylic acids (8–10 carbons) and monocarboxylic acids (10–12 carbons). However, overlap between the individual receptive ranges suggests that the members of this subfamily form one contiguous subfamily receptive range, suggesting that odorant receptor subfamilies do constitute functional units. It was found that the ligand specificity of MOR174-9 (mOR-EG) expressed in oocytes (activation by eugenol and antagonism by methyl isoeugenol) recapitulates the ligand specificity of this receptor when expressed in olfactory neurons (Oka et al. [48]). It was also assessed that the ligand specificities of MOR42-1 and MOR42-3 expressed in oocytes agree well with the properties of these receptors expressed in olfactory neurons (Malnic et al. [41]). A related concern that is particularly important for olfaction is the observation that functionally characterized ORs seem much less

sensitive than might be expected, given the extraordinary sensitivity of mammalian olfaction (Mombaerts [49]). In this work, the most potent ligands for MOR42-1 and MOR42-3 activate these receptors with EC50s in the low micromolar range, and ligand sensitivities in the low to mid micromolar range are common for other ORs, whether expressed in heterologous cells (Kajiya et al. [50]; Saito et al. [51]) or isolated olfactory neurons (Touhara et al.[52]; Bozza et al. [53]; Oka et al.[48]).

Using the Xenopus oocyte system the receptive range of all members of one MOR subfamily were examined. The closely related MOR42-1 and MOR42-3 have overlapping ligand specificities, but can distinguish among odorants based on small structural features. The general requirements for agonists of these receptors are similar. Their results provide insight into the participation of an OR subfamily in the combinatorial coding of odorant recognition.

Although each receptor in this subfamily recognizes a unique range odorants, these receptive ranges overlap, with some odorants being recognized by two receptors. Thus, the individual receptors appear to be contributing to one contiguous subfamily receptive range. These results support the proposal that OR subfamilies constitute functional units (Zhang et al. [46]; Godfrey et al. [54]) [47].

Further research in subfamily of OR has shown that sequence differences between members of the mouse olfactory receptor MOR42 subfamily (MOR42-3 and MOR42-1) are likely to be the basis for variation in ligand binding preference among these receptors. Abaffy and al. [55] have investigated the specificity of MOR42-3 for a variety of dicarboxylic acids using the site-directed mutagenesis, guided by homology modeling and ligand docking studies. They have identified eight residues that participate in determining the ligand specificity of MOR42-3. These residues are located in TMs III, V and VI. The most structurally and functionally important of these residues is valine 113. The importance of the V113 residue, located deep within the receptor, was analyzed in the context of interhelical interactions. They have also screened additional residues predicted to be involved in ligand binding site, based on comparison of ortholog/paralog pairs from the mouse and human olfactory receptor genomes. C12 dicarboxylic acid did not activate the receptor in functional assay, yet docking simulations predicted its binding site in MOR42-3. Binding without activation implied that C12 dicarboxylic acid might act as an antagonist. In functional assay, C12 dicarboxylic acid did indeed act as an antagonist

of MOR42-3, in agreement with molecular docking studies. This results demonstrate a powerful approach based on the synergy between computational predictions and physiological assays [55].

Dual functions of odorants as an agonist and an antagonist to ORs indicate a new aspect in the receptor code determination. The study from Oka et al. [48] provides insight into strategies to modulate perceived odorant quality. The possibility of antagonism at the level of ORs has also been suggested for other mammalian ORs (Araneda et al. [56]; Spehr et al. [57]). Despite increasing information on agonist, OR combinations, little is known about the antagonism of ORs. In this study, authors provided molecular and cellular evidence for the antagonism of OR activities between odorants. They showed that odorants inhibit odorant responses of OR(s), an evidence of antagonism between odorants at the receptor level. The antagonism was demonstrated in a heterologous OR-expression system and in single olfactory neurons that expressed a given OR, and was also visualized at the level of the olfactory epithelium. Pharmacological analyses of receptor antagonism in HEK293 cells and single olfactory neurons that expressed a defined OR clearly demonstrated that odorant mixture suppression occurred at the receptor level.

Deducing a structure–activity relationship from profiles of agonists and antagonists of ORs will definitely stimulate the field of drug discovery. Having a repertoire of agonists and antagonists, a combination of computational and mutational strategies will enable to predict a ligand-binding site and to elucidate molecular bases for ligand discrimination. Further, examining an antagonist-bound form that represents the inactive state will provide insight into a molecular basis for agonist-induced conformational changes of GPCRs. Extensive analysis of ligand specificity has also been carried out for the rat I7 odorant receptor, showing that the I7 receptor recognized octanal as a primary agonist, and its structurally similar odorant, citral, behaved as a partial agonist or antagonist (Araneda et al. [56]). An inhibitor for human OR17-4 was identified to be undecanal, which possesses an aldehyde group as a common functional group with the ligands, bourgenonal and cyclamal (Spehr et al. [57]). These reports and the current study demonstrated that antagonists tend to be structurally related to the agonists, as is often the case for other GPCRs. Together with the previous electrophysiological and biochemical studies, molecular evidence

for the peripheral OR antagonism provides one of the likely explanations for odorant mixture interactions leading to novel perceptual qualities of odorant mixtures [48].

Zou et al. [58] have studied a combinatorial effects of odorant mixtures in olfactory cortex. In mammals, each odorant is detected by a combination of different odorant receptors. In this study they have reported that binary odorant mixes stimulate cortical neurons that are not stimulated by their individual component odorants. They proposed that cortical neurons require combinations of receptor inputs for activation and that merging the receptor codes of two odorants provides novel combinations of receptor inputs that stimulate neurons beyond those activated by the single odorants. These findings may explain why odorant mixtures can elicit novel odor percepts in humans. Neurons stimulated by an odorant mix, but not its individual components, are those that receive novel combinations of OR inputs that result from merging the receptor codes of two odorants. This would represent a synthetic operation in which the deconstructed features of an odorant, which are carried by different OR inputs, begin to be reconstructed at the level of individual cortical neurons in order to generate a unique odor perception. The present studies suggest that these mixture effects may be due to the novel cortical representations that result from mixing odorants. Given that most natural odors derived from complex blends of odorants, it is quite possible that they emerge from cortical representations that bear only a remote resemblance to those of their component odorants [58].

In order to initiate the process of determining how the molecular level receptor-odorant interactions are related to odor perception, Hall et al. [59] have used the MembStruk computational method to predict the three-dimensional (3-D) structure of the I7 OR for both mouse and rat. Further more, the HierDock ligand docking computational method to predict the binding site and binding energy for the library of 56 odorants to these receptors were used for which experiment response data are now available. A group of scientists have found that the predicted 3-D structures of the mouse and rat I7 OR lead to predictions of odorant binding that are in good agreement with the experimental results, thus validating the accuracy of both the 3-D structure and the predicted binding site [59].

Continued progress in the understanding of olfactory receptor function has been significantly hampered by the difficulty in expressing ORs in heterologous cells. Neuhaus et al. [60] found the testisenriched HSP Hsc70t in a proteomic analysis of

mouse olfactory epithelium and confirmed the expression in human and mouse olfactory epithelium by RT–PCR. Due to the fact that olfactory receptors are expressed in the human (Spehr et al. [57]) and mouse (Fukuda et al. [61]) sperm, they have speculated that a testis and olfactory epithelium chaperone might contribute to folding or cell-surface targeting of OR proteins.

In this paper it was demonstrated that Hsc70t plays a role in functional expression of ORs and can thereby help to identify ligands for orphan ORs. Hsp70s assists folding processes, assembling of newly synthesized proteins, refolding of misfolded and aggregated proteins, membrane translocation of proteins, and control of the activity of regulatory proteins (Young et al. [62]). Hsp70 was found to be predominantly localized to the sustentacular cells, basal cells, and Bowman's glands of the olfactory epithelium (Simpson et al. [63]). Odorant exposure, heat shock, or toxic chemicals lead to a transient induction of Hsp70, Hsc70, Hsp25, and ubiquitin immunoreactivities in supporting cells and Bowman's gland acinar cells, but not in ORNs (Carr et al. [64]; Simpson et al. [63]). Hsp70 has also been localized in human ORNs and in a subpopulation of rat ORNs (Carr et al. [65]). Hsc70t promotes the heterologous expression of OR proteins, but the exact mode as well as time and place of action during the synthesis and transport of the OR protein are still largely unclear [60].

In this study Man et al. [66] predict the binding site residues of OR proteins by analyzing a set of 1441 OR protein sequences from mouse and human. Using judiciously selected subsets of 218 ortholog pairs and 518 paralog pairs, they have identified 22 sequence positions that are both highly conserved among the putative orthologs and variable among paralogs. These residues are disposed on transmembrane helices 2 to 7, and on the second extracellular loop of the receptor. Although the prediction makes no assumption about the location of the binding site, these amino acid positions are clustered around a pocket in a structural homology model of ORs, mostly facing the inner lumen [66].

Further research have shown that the experimentally determined odorant-binding site confers the broad but selective ligand spectrum of the G-protein-coupled OR superfamily. Katada et al. [67] have found that most of the critical residues involved in odorant recognition, and therefore sensitive to mutation, were hydrophobic and that the binding pocket was in the space formed by TM3, TM5, and TM6. Based on

the effects of mutations on antagonist activity, they have identified an amino acid in TM6 that was involved in receptor dynamics involved in transition from an inactive to an active conformation. The combination of functional experimental analysis and computational docking simulation strongly suggests the molecular basis of the structure–activity. The results indicate that several amino acids in the transmembrane domains formed a ligand-binding pocket. Although other G-protein coupled receptors (GPCRs) recognize biogenic ligands mainly with ionic or hydrogen bonding interactions, ORs recognize odorants mostly via hydrophobic and van der Waals interactions. Furthermore, they succeeded in rational receptor design, inserting point mutations in the odorant-binding site that resulted in predicted changes in ligand specificity and antagonist activity. This ability to rationally design the receptor validated the binding site structure that was deduced with mutational and ligand docking studies. Such broad and specific sensitivity suggests an evolutionary process during which mutations in the active site led to an enormous number of ORs with a wide range of ligand specificity [67].

The large number of olfactory receptor genes necessitates high throughput methods to analyze their expression patterns. In these studies Zhang et al. [68] have designed a high-density oligonucleotide array containing all known mouse olfactory receptor (OR) and V1R vomeronasal receptor genes. Their main findings were that the custom array reliably detects a large number of OR transcripts, that most OR genes are preferentially expressed in the olfactory epithelium, that OR genes undergo developmental regulation, that spatial expression patterns in the OE are reflected in chromosomal organization, and that OR genes distribute unequally between zones. This custom array detected a large number of receptor genes, demonstrating specific expression in the olfactory sensory epithelium for 800 OR genes previously designated as ORs based solely on genomic sequences. The array also enabled to monitor the spatial and temporal distribution of gene expression for the entire OR family. OR genes showing spatially segregated expression patterns were also segregated on the chromosomes. This correlation between genomic location and spatial expression provides unique insights about the regulation of this large family of genes. Examination of the 10 zonal samples by hierarchical clustering showed that OR genes can be roughly separated into three categories: those enriched in dorsal samples, those enriched in ventral samples, and those without apparent enrichment in

either. This observation prompted them to use an unsupervised clustering method, K-means clustering, to separate OR genes into groups with a high degree of similarity within each group and a low degree of similarity between groups [68].

In the studies from Fingueroa et al. [69] they have used microwave array in investigation of the OR space. In mice, each odorant is sensed by a small subset of the approximately 1000 odorant receptor (OR) types, with one OR gene expressed by each olfactory sensory neuron (OSN). The sum of the large repertoire of OR-OSN types and difficulties with heterologous expression have made it almost impossible to analyze odorant-responsiveness across all OR-OSN types. They have developed a microfluidic approach that allowed to screen over 20,000 single cells at once. By using calcium imaging, they were able to detect and analyze odorant responses of about 2900 OSNs simultaneously. This technique is generally applicable for screening large numbers of single cells and should help to characterize rare cell behaviors in fields such as toxicology, pharmacology, and cancer research [69].

Recent evidence has revived interest that phosphoinositides (PIs) may play a role in signal transduction in mammalian olfactory receptor neurons (ORNs). To provide direct evidence that odorants indeed activate PI signaling in ORNs, Klassen et al. [70] have used adenoviral vectors carrying two different fluorescently tagged probes, the pleckstrin homology (PH) domains of phospholipase Cδ1 (PLCδ1) and the general receptor of phosphoinositides (GRP1), to monitor PI activity in the dendritic knobs of ORNs *in vivo*. Then they have measured odorant activation of PLC and PI3K in olfactory ciliary-enriched membranes *in vitro* using a phospholipid overlay assay and ELISAs. Odorant-dependent activation of PLC and PI3K in the olfactory epithelium could be blocked by enzyme-specific inhibitors. These results provide direct evidence that odorants indeed activate PI signaling in mammalian ORNs in a manner that is consistent with the idea that PI signaling plays a role in olfactory transduction [70].

Wilson [71] described olfaction as a model system for the neurobiology of mammalian short-term habituation. Further, this review has emphasized mechanisms of short-term habituation, recent behavioral pharmacology work has demonstrated a double-dissociation between short- and long-term odor habituation, with long-term habituation of odor investigation relying on an NMDA receptor-dependent mechanism, and as described here, short-term habituation relying on an mGluRIII

mechanism (McNamara et al. [72]). Together with the known relative simplicity of the olfactory sensory pathway, these findings place olfaction as an ideal model system for the study of the neurobiology of mammalian habituation [71].

The mammalian olfactory system detects an unlimited variety of odorants with a limited set of odorant receptors. To cope with the complexity of the odor world, each odorant receptor must detect many different odorants. The demand for low odor selectivity creates problems for the transduction process: the initial transduction step, the synthesis of the second messenger cAMP, operates with low efficiency, mainly because odorants bind only briefly to their receptors. Sensory cilia of olfactory receptor neurons have developed an unusual solution to this problem. They accumulate chloride ions at rest and discharge a chloride current upon odor detection. This chloride current amplifies the receptor potential and promotes electrical excitation. Hengl et al. [73] have studied this amplification process by examining identity, subcellular localization, and regulation of its molecular components and they found that the $Na^+/K^+/2Cl^-$ cotransporter NKCC1 is expressed in the ciliary membrane, where it mediates chloride accumulation into the ciliary lumen. Gene silencing experiments revealed that the activity of this transporter depends on the kinases Ste-20 related proline alanine-rich kinase (SPAK) and Oxidative stress response kinase (OSR1), which are enriched in the cilia together with their own activating kinases, With-no-lysine kinase (WNK) WNK1 and WNK4. A second Cl^- transporter, the Cl^-/HCO_3^- exchanger "solute carrier family 4, anion exchanger, member 1" SLC4A1, is expressed in the cilia and may support Cl^- accumulation. The calcium-dependent chloride channel provides a ciliary pathway for the excitatory chloride current. These findings describe a specific set of ciliary proteins involved in anion-based signal amplification. They provide a molecular concept for the unique strategy that allows olfactory sensory neurons to operate as efficient transducers of weak sensory stimuli [73].

Both vertebrates and insects have receptors for detecting odor molecules in the environment, but the evolutionary origins of these genes are different. Mammals have 1,000 olfactory receptor (OR) genes, whereas fishes have much smaller (100) numbers of OR genes. To investigate the origin and evolution of vertebrate OR genes, Niimura [74] has attempted to determine near-complete OR gene repertoires by searching whole-genome sequences of 14 nonmammalian chordates, including

cephalochordates (amphioxus), urochordates (ascidian and larvacean), and vertebrates (sea lamprey, elephant shark, five teleost fishes, frog, lizard, and chicken), followed by a large-scale phylogenetic analysis in conjunction with mammalian OR genes identified from nine species. This analysis showed that the amphioxus has >30 vertebrate-type OR genes though it lacks distinctive olfactory organs, whereas all OR genes appear to have been lost in the urochordate lineage. Some groups of genes that are phylogenetically nested within vertebrate OR genes showed few gene gains and losses, which is in sharp contrast to the evolutionary pattern of OR genes, suggesting that they are actually non-OR genes. The analysis demonstrated a great difference in OR gene repertoires between aquatic and terrestrial vertebrates, reflecting the necessity for the detection of water-soluble and airborne odorants [74].

In studies from Wetzel et al. [75] about cellular mechanisms of olfactory signal transduction, their interest is to understand the molecular and cellular mechanisms of chemosensation in the diverse subsystems. In this work they have been focused on the cellular and molecular biology of chemosensory transduction in olfactory sensory neurons (OSNs) of rodents. The data suggest that at least in a subpopulation of OSNs certain odorants can induce the activation of the phosphatidyl-inositol 3-kinase (PI3K) pathway and the generation of phosphatidyl-inositol-3,4,5-trisphosphate (PIP3) which is acting as a signalling molecule. To establish a second line of evidence they have extended their studies to electrophysiological investigation of dissociated mouse OSN by whole-cell voltage-clamp recordings. It could be proved that PIP3 is able to inhibit odorant or forskolin-induced calcium signalling, suggesting that PIP3 is acting at the level or downstream of the adenylyl cyclase. Conducting the electrophysiological experiments it could be shown that complex odorants can both activate and inhibit the transduction current and that the inhibition could be abolished by the action of the PI3K inhibitor. From that the scienties can conclude that PIP3 is acting directly at the CNG channel or at upstream targets. These results are in favor of the hypothesis that individual OSNs can detect different odorant molecules which are able to stimulate an excitatory and inhibitory pathway in the same cell [75].

By measuring the fluorescence of intrinsic tryptophan and tyrosine residues of intact odorant binding protein (OBPb) and OBPb whose C-terminal 10 amino acids were

deleted, it was clarified that an odorant enters the central pocket formed by the dimerization when OBPb first encounters the odorant, and odorants with high affinity with OBPb subsequently enter the internal cavity, releasing the pre-bound odorant. The internal cavity-bound odorant can be released by the binding of other odorants at another internal cavity or at the central pocket, depending on the binding odorants. Due to this mechanism enabled by the dimerization, OBPb (bovine) is more reactive than other monomeric OBPs. These results suggest a simplified binding model OBPb-dimer eventually monomerizes when no odorants are bound but is stabilized when odorants are bound [76].

It has been suggested that positive selection plays a role in the evolution of olfactory receptor (OR) gene repertoires in fish and mammals. OR gene repertoires in birds are surprisingly large and diverse, suggesting that birds have a keen olfactory sense. The aim of the study from Streiger et al. [77] is to investigate signatures of positive selection in an expanded OR (group-gamma-c) that seems to be a characteristic of avian genomes in bird species. Positively selected codons were predominantly located in TMs, which in other vertebrates are involved in odorant binding. The data suggest that at least some avian OR genes have been subjected to adaptive evolution, the extent of such adaptive evolution differs between bird species, and positive selective pressures may have been stronger on the group-gamma-c OR genes of species that have well-developed olfactory abilities [77].

The *Drosophila* antenna is a highly derived appendage required for a variety of sensory functions including olfaction and audition. *Drosophila* is a genus of small flies, belonging to the family Drosophilidae, whose members are often called "fruit flies". One species of *Drosophila* in particular, *D. melanogaster*, has been heavily used in research in genetics and is a common model organism in developmental biology.

Odor coding in the Drosophila antenna is examined by a functional analysis of individual olfactory receptor neurons (ORNs) *in vivo*. Sixteen distinct classes of ORNs, each with a unique response spectrum to a panel of 47 diverse odors, are identified by extracellular recordings. ORNs exhibit multiple modes of response dynamics: an individual neuron can show either excitatory or inhibitory responses, and can exhibit different modes of termination kinetics, when stimulated with different odors. The 16 ORN classes are combined in stereotyped configurations

within seven functional types of basiconic sensilla. One sensillum type contains four ORNs and the others contain two neurons, combined according to a strict pairing rule. De Bruyne et al. [78] have provided a functional map of ORNs, showing that each ORN class is restricted to a particular spatial domain on the antennal surface [78].

The functional overlap among receptors expands the coding capacity of the system by allowing for combinatorial coding, which has been documented previously in other systems (Malnic et al. [41]; Kajiya et al. [50]).

Hallem et al. [79] have found that coding capacity is further expanded. They have shown that receptors confer not only the odor response spectrum but also the response mode and the response dynamics upon the ORNs that express them, as well as the level of spontaneous activity [79].

Schmuker et al. [80] have demonstrated that it is possible to predict *Drosophila* ORN responses from molecular structure. The ORN responses themselves can effectively be used as a descriptor to predict responses of other ORNs, providing evidence that ORNs indeed analyze chemical space in a way that can be exploited to predict receptor-ligand affinities. The authors have used the molecular modeling software package Molecular Operating Environment (MOE) (Chemical Computing Group, Montreal) for each odorant molecule to calculated 203 molecular descriptors. Prior to descriptor calculation, they have generated heuristic 3D conformations with "CORINA" (Molecular Networks, Erlangen, Germany). These models were tested by recording *in vivo* receptor neuron responses to a new set of odorants and successfully predicted the responses of five out of seven receptor neurons. The molecular descriptors that are best-suited for response prediction vary for different receptor neurons, implying that each receptor neuron detects a different aspect of chemical space. The scientists have demonstrated that receptor responses themselves can be used as descriptors in a predictive model of neuron activation [80].

Odorant receptors (ORs) in insects, such as *Drosophila melanogaster*, have long been thought to belong to the G-protein coupled receptor (GPCR) superfamily. Recent work has cast doubt on this assumption and has tentatively suggested an inverted topology compared to the canonical N(out)-C(in) 7 transmembrane (TM) GPCR, at least for some *Drosophila* ORs. Lundin et al [81] have reported a detailed topology mapping of the *Drosophila* OR83b receptor using engineered glycosylation

sites as topology markers, an approach that has been widely applied to eukaryotic membrane proteins. Their results are inconsistent with a classical GPCR topology and show that OR83b has an intracellular N-terminus, an extracellular C-terminus, and 7TM helices. The *Drosophila* OR83b protein is an ubiquitously expressed member of the insect OR family, and it forms functional heteromers with other OR proteins. Mammalian ORs are 7TM GPCRs with an EC N terminus, but there is no detectable sequence similarity between mammalian and insect ORs [81].

In `Molecular Basis of Odor Detection in Insects`, Benton [82] has discussed recent investigations of ORs in the fruit fly, *Drosophila melanogaster*, which have revealed insights into the distinct evolutionary origin and molecular function of insect ORs. In addition he describes a bioinformatics strategy that his group developed to identify molecules that function with these insect-specific receptors in odor detection [82].

Each down stroke of an insect's wings accelerates axial airflow over the antennae. Modeling studies suggest that this can greatly enhance penetration of air and air-born odorants through the antennal sensilla thereby periodically increasing odorant-receptor interactions. Tripathy et al. [83] have monitored antennal and antennal lobe (AL) responses in the moth *Manduca sexta* while odorants were pulsed at frequencies from 10–72 Hz, encompassing the natural wingbeat frequency. Power spectral density (PSD) analysis was used to identify entrainment of neural activity. Psychophysical measures of odor detection established that detection thresholds are lowered when odor is pulsed at 20 Hz. These results suggest that AL networks can respond to the oscillatory dynamics of stimuli such as those imposed by the wing beat in a manner analogous to mammalian sniffing.

This is consistent with current behavioral and physiological results in mammals, which indicate that odor discrimination can occur within one or two sniffs. Both population analytic (Daly et al. [84]; Brown et al. [85]) and behavioral (Ditzen et al. [86]; Budick et al. [87]) results in insects suggest that the time to process odor cues is on the order of 250 ms. This does not imply that a 250-ms stimulus is required. Rather, this is the time for transduction, processing and initiation of the behavioral response; successive cycles are likely being processed simultaneously at different points in the pathway [83].

4. Odors

4.1. Sandalwood

Sandalwood products are obtained from the sandalwood tree (*Santalum album*), which is a member of the Santalaceae family. Originally derived from the heartwood and the roots of *Santalum album* trees grown in India and Indonesia, sandalwood oil comprises more than 100 odoriferous compounds. The main constituents are α-santalol and β-santalol. α-Santalol represents up to 50% of natural sandalwood oil and has a strong woody, cedarwood-like odor. β-Santalol, which contributes up to 30% to the essential oil, imparts the typical sandalwood note including powerful woody, milky, and urinous tonalities.

Sandalwood oil is best known as a sweet, warm, rich and woody essential oil that promotes a feeling of well-being used for a body fragrance, and as an ingredient in fragrant products such as incense, perfumes, aftershaves and other cosmetics. Sandalwood is a relaxant with sensual properties, that is why it has found use in aromatherapy, perfumery, in spiritual tradition. The oil is used for all types of skin care. It may also be used in the treatment of bronchitis, depression, laryngitis, scars, and stress. Its main component β-santalol has antimicrobial properties. Because of its strength, sandalwood oil should never be applied to the skin without being diluted in a carrier oil [84].

The current production of sandalwood trees is not enough to meet the demand of consumers. The trees are difficult to propagate and must grow for at least 30 years to become suitable for harvesting. The situation regarding sandalwood trees is getting worse and this divine wood and the oil from it are becoming more and more precious. In the west, it is needed to look for ways to responsively use this resource and to reduce our dependence on it [85].

Natural sandalwood oil, a unique and valuable ingredient in fine perfumery, has been the focus of scientific interest for many years. Due to its scarcity and its high price, the search for novel synthetic raw materials imitating the characteristic odor profile of sandalwood oil is as challenging as ever. The search for synthetic substitutes has become, since several decades, the subject of various worldwide research activities.

Chapuis [86] has compared optical antipodes of (-)-β- and (+)-α-santalol, thus assuming a very similar concentration at the receptor level, since they possess identical physical properties. The task was restrained to the comparison of the 3D interactions between the ligands and the receptor(s). Based on similarities between naturally occurring (-)-β- or (+)-α-santalol and the reversed (*E*)-configurated synthetic derivatives from campholenal, a simple model was developed. A versatile starting material, available in both antipodal forms and subject to various possible substitutions in the lipophilic and/or polar part, is ideally represented by campholenic aldehyde. By structurally targeting and constraining the bioactive conformation of sandalwood-like odorants, several models have been accommodated in a common simple structural network evolving from the trans-decalinic to the bicyclo[2.2.2]octane system. Both trimethylcyclopentene lipophilic parts of (*R*)- and (*S*)-enantiomeric series of analogues derived from campholenic aldehyde agree well with these models. The lipophilic region is characterized by three adjacent potential quarternary centers, highly substituted center and the OH osmophore.

Besides reconciliation of stereochemical aspect, the model in this study also tentatively explained the enantiodiscriminations as well as the large spectra of distances separating the OH function from the lipophilic quaternary center(s) reported for different classes of substrates [86].

The ampirical rules were also used in resarch of sandalwood and camphoraceous odors application. In this paper structure–odor relationships for sandalwood odorants were studied for a set of 158 compounds (75 inactive and 83 active). The first step consists in an evaluation of empirical rules concerning structure–sandalwood odor relationships. The main structural elements responsible for other than sandalwood odors are defined from the empirical rules. The rules used to discriminate between sandalwood and non-sandalwood molecules lead to 80% correct discrimination. Fuzzy logic has been also used as a tool in structure–camphoraceous odor relationships. The database studied included 99 molecules. The rules used to discriminate between camphor and non-camphor are given by an expert. Such rules account for the shape and the size of the molecules. Their adjustment by means of genetic algorithms (GAs) lead to 84% correct discrimination between camphor and non-camphor molecules [87].

Quantitative structure-activity relationship of α-campholenic derivates with sandalwood odor was a subject of research in study by Kovatcheva et al. [88]. (3D-QSAR) models were developed for a series of 44 synthetic alpha-campholenic derivatives with sandalwood odor. The data set was divided into two training sets: one of 38 compounds and a second one of six compounds. These compounds have complex stereochemistry, they contain up to five chiral atoms. In QSAR method, compounds are represented as derivatives of several common structural templates with several substituents which axe numbered according to their relative spatial positions in the molecule. Both wholistic and substituent descriptors calculated with the TSAR software were used as independent variables. To build QSAR models a stepwise multiple linear regression method was used. The best model was obtained using the unequal scale of odor intensity. The QSAR models developed in this study contribute to the better understanding of structural, electronic, and lipophilic properties responsible for sandalwood odor [88].

In 2005, Kovatcheva et al. [89] have discussed the development and validation of several approaches for describing chiral descriptors in the context of computationally efficient and robust QSAR modelling. They have developed simple, alignment free chirality as well cis-trans isomerism descriptors that afford robust and predictive QSAR models for the datasets with enantiomers. In their studies they used two enantiospecific data sets: 44 sandalwood compounds and 98 ambergris chiral molecules of several structural types [89].

Recent studies have provided evidence for the code of smell, but all these findings only scratch at the surface of the paradigm to understand the sense of smell (Krautwurst [28]; Malnic et al. [41]; Araneda et al. [7], [56]; Kajiya et al. [50]; Bozza et al. [53]; Spehr et al. [6]; Oka et al. [48]). The study from Bieri et al. [90] 'Olfactory Receptor Neuron Profiling using Sandalwood Odorants' was one of the first study in which an important class of perfume compounds was analyzed for its ability to activate endogenous olfactory receptors in olfactory receptor neurons. Sandalwood oil and four synthetic sandalwood molecules: Sandalore®, Ebanol®, Radjanol® and Javanol®, were selected to study the activation profile of endogenous olfactory receptors when exposed to compounds from the same odorant family. Dissociated rat olfactory receptor neurons were exposed to the sandalwood molecules and the receptor activation studied by monitoring fluxes in the internal

calcium concentration. These neurons expressed olfactory receptors that can discriminate between sandalwood odorants with slight differences in their molecular structures [90].

In the study of Hölscher et al. [91] the enantioselective total synthesis of a new class of sandalwood odorants Fleursandol® was reported. Tricyclo[5.2.1.$0^{2,6}$]decene as building block A and butanol derivatives B was used as starting materials. The configuration of the side-chain especially at position C(2) is important for the odor intensity. These results are in excellent agreement with previous findings both for Ebanol and Polysantol, where also the most intense isomer has (*S*)-configuration at side-chain position C(2). The multifaceted odor of Fleursandol (rac-10) is very reminiscent to East-Indian sandalwood oil [91].

In the following studies a group of scientists has investigated the structure-activity relationships of sandalwood odorants of β-santalol analogues.

The synthesis and odor properties of a new santalol analogue, cyclopropano-β-santalol, are described in the study of Stappen et al. [92]. In this model the exocyclic double bond of the original molecule is replaced by a cyclopropane ring. Despite the analogies in the binding properties between the double bond and cyclopropane this change in the bulky hydrophobic part of the molecule leads to the complete loss of the characteristic sandalwood odor: in an olfactory evaluation the (*Z*)-product appears spicy and sweet, the (*E*)-isomer woody, but neither of them exhibits the typical sandalwood character. That shows the sensitivity of sandalwood odor on the shape of the hydrophobic, bulky part of β-santalol analogues [92].

In 2004 they described a structure–odor relationship and the multi-step synthesis of a new tricyclic β-santalol derivative. The modification with a bulky aliphatic bridge in the neighbourhood of the quaternary C3-atom demonstrated the sensitivity of sandalwood odor on the structure of β-santalol analogues [93].

Further research in this area has shown that three osmophoric points are necessary for the scent of sandalwood odorants, like the bulky group in a certain distance from the osmophoric hydroxyl group. Such a hydrophobic moiety is part of the trimethylcyclopentenyl derivatives, called campholenals, with a strong and long lasting sandalwood odor. In continuation four isophorone analogues of β-santalol have been synthesized. The hydrophobic region of these new isophorone derivatives

is now a trimethylcyclohexene nucleus. This modification changes the sandalwood odor drastically to woody odor notes, only reminiscent to sandalwood odor [94].

C-13-NMR spectra of a systematic investigation of a series of santalol and epi-santalol derivatives were studied by Stappen et al. [95]. In addition *ab initio* and density functional theory (DFT) calculations together with database-oriented prediction methods were used. The DFT calculations as well as the HOSE-code and neural network-based predictions allow deriving a general rule set for unambiguous assignment within this compound class. The methyl group in position 2′ allowes easy differentiation between santalol derivatives and their diastereomers belonging to the epi-santalol series [95].

Several new and differently functionalized cis-2,3-dimethylnorbornane derivatives with diverse side-chain lengths were prepared from Muratore et al. [96]. The structures are related to the natural fragrance β-santalol. In particular, exo- and endo-3,8-dihydro-β-santalols, with either (*E*)- or (*Z*)-C=C-bond configuration on the side chain, were synthesized in seven steps and 21-24% overall yields. Several other exo- and endo-norbornyl alcohols with shorter side chains were also prepared in high yields. The olfactory evaluation indicated woody, sandalwood, as well as fruity notes for some of the derivatives [96].

Castro et al. [97] synthesized enantiospecifically the sandalwood odorant Polysantol®. The four stereoisomers of (5*E*)-4,4-dimethyl-6-(2′,2′,3′ trimethylcyclopent-3′-en-1′-yl)-hex-5-en-3-ol were enantiospecifically synthesized from (+)- and (−)-α-pinene, through (−)- and (+)-campholenic aldehyde, by aldol condensation with 3-pentanone, deconjugative α-methylation and reduction [97].

Further studies on Polysantol® were made. Chapado et al. [98] investigated the influence of the global shape of the hydrophobic moiety C and the olfactophore model on compounds structurally similar to Polysantol®. Five new bulky moiety modified analogues of the commercial sandalwood odorant Polysantol® have been synthesized and their odor evaluated. The independent odor evaluation of each bulky moiety-modified Polysantol® analogue (each over 97% pure according to GC) was carried out by a group of perfumers. Thus, the profile of the (*E*)-3,3-dimethyl-5-((1*S*,2*S*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-yl)pent-4-en-2-ol was identified as the most interesting and promising of the series because it is full of qualities and it

directly emulates the natural sandalwood odor instead of that of synthetic Polysantol®. This compound has been claimed as a potential useful odorant [98].

Brocke et al. [99] have searched for novel synthetic raw materials mimicking all aspects of the broad olfactory spectrum of sandalwood oil. The aim was to investigate the influence of the substitution pattern of the cycloaliphatic spacer on the sandalwood olfactophore. The synthesis of new cyclohexanol and cyclohexenol derivatives with substituents attached to different positions of the ring system was envisaged. A calculated structure of the novel sandalwood odorants is depicted. The three new sandalwood compounds have been prepared by careful consideration of the structures of several well-known sandalwood odorants. These results clearly indicate that the establishment of structure–odor relationships is a powerful tool for the generation of novel fragrant molecules. Regarding the osmophore, in case of the novel odorants the polar OH group is an allylic alcohol function such as present in the natural sandalwood oil constituents (*Z*)-α-santalol and (*Z*)-β-santalol, as well as in the synthetic sandalwood odorants Madrol® and Sandranol®. Sticking out of the plane established by the cyclohexenyl ring like an anchor, the spatial orientation of the OH groups in new derivates is very similar to the minimum-energy conformation calculated for the structure of the natural (Z)-β-santalol [99].

Cheng et al. [100] presented the results of a study, based on quantum chemical calculation methods, of structural and electronic features of some terpenylcyclohexanols with sandalwood odor. The method that was used made it possible to achieve the clearest and most complete isolation of the structural fragment with definite geometric and electronic properties responsible for sandalwood odor. Cheng et al. have investigated the effects of HOMO–LUMO energy gaps and total energies of some terpenylcyclohexanols on their odor intensity. Geometry optimization and electronic structure analysis revealed that all active sandalwood compounds have an activity fragment which is absent from the inactive compounds. The activity fragment consists of one oxygen and three hydrogen atoms. The four atoms must make a major contribution to the HOMO of the molecule or to an occupied orbital lying close to the HOMO [100]. The quantum chemical calculation method was also used in studies of amber (see also [95]).

Pick et al. [101] have published a study of dual activities of odorants on olfactory and nuclear hormone receptors. They have screened an odorant compound library

with diverse chemical structures for their potential to activate ER and discovered molecules acting as chemical signals that specifically activate both G-protein-coupled olfactory receptors (ORs) on the cell surface of olfactory sensory neurons and the human nuclear estrogen receptor alpha (ER) involved in transcriptional regulation of cellular differentiation and proliferation in a wide variety of tissues. The authors demonstrated these effects using fluorescence-based *in vitro* and cellular assays. Among these odorants, they have identified synthetic sandalwood compounds and for one estrogenic odorant they have also identified the cognate OR.

In addition, the evidence that certain olfactory sensory neurons naturally co-express ORs and ERs may provide a direct functional link between the olfactory and hormonal systems in humans. Most potent for activating ER were methyl 2,4-dihydroxy-3,6-dimethylbenzoate (also known as Mousse Cristal® (MC)) and molecules of the sandalwood odorant family, which are extensively used in modern perfumery as synthetic substitutes of the sandalwood oil. This is the first demonstration that sandalwood-derived odorants act as ER-specific agonists. Pick et al [101] have also confirmed the estrogenic activity of the odorant molecules by measuring their proliferative effect on the division of MCF7 human breast cancer cells [101].

Carbin et al. [102] declared that Sandalwood oil shows anticarcinogenic, antiviral and bactericidal activity. Cases of irritation or sensitization reactions to sandalwood oil in humans were reported. Although the available information on toxicity of sandalwood oil is limited, it has a long history of oral use without any reported adverse effects and is considered safe at present use levels [102].

Santalols in medium and/or high concentrations in sandalwood oils show a significant influence on antimicrobial potential in natural products. Jirovetz et al [103] have taken eight samples of different sandalwoods and a mixture of α- and β-santalols, as well as eugenol as reference compound, and tested these samples for their antimicrobial activities against the *Candida albicans*, *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* For the santalol mixture, as well as for one *S. album* and one *S. spicatum* sample with moderate concentrations of santalols, antimicrobial activity was found against all the strains used [103].

4.2. The woody-ambery odorant Georgywood

Georgywood was found to be one of best odorants that shows the same odor threshold and possessing a very attractive warmwoody, sweet-powdery smell. The first synthesis of the olfactorily active (-)-(1*R*,2*S*)- enantiomer of Georgywood has been accomplished via classical racemate resolution. Conformational studies and CD measurements allowed the determination of the absolute configuration. The much lower olfactory threshold in comparison to its antipode renders the (-)-enantiomer as a promising candidate for fragrance applications, provided an industrially feasible and inexpensive asymmetric synthesis can be developed. Conversion to the final ketone and olfactory evaluation showed that the (-)-(1*R*,2*S*)-enantiomer is more powerful by a factor of >100 than its antipode [104].

Doszczak et al. [105] have discovered that α-[dimethyl(hexyl)siliyl]acetaldehyde has a strong woody odor. On the basis of structure-odor-relationships, new and more powerful woody and ambery sila odorants were prepared which may find application of organosilicon compounds in the fragrance and flavor industry.

Further derivatization led to a set of compounds with a very interesting palette of organoleptic properties. A simplified general model for ozone-like odorants was designed and together with a similar model of sandalwood odorants was employed in a design of new sila odorants [105].

In the acid-promoted 1,5-diene cyclization of pseudo- to β-Georgywood, the cyclization product is obtained with high selectivity in spite of an unfavorable substituent at the C(2)-position of the diene precursor. Mechanistic studies revealed a crucial participation or nonparticipation of the carbonyl group in the cyclization reaction, depending on the acid family employed, and allowed finally the development of a cyclization reaction catalyzed by $MeAlCl_2$ that can be generated *in situ* from precatalyst $AlMe_3$ [106].

In this study a synthesis of octahydronaphthalene-based fragrance, such as Georgywood, is described. The octahydronaphthalene skeleton constitutes a structural requirement of industrially significant fragrances, such as Iso E Super, its powerful minor constituent, and Georgywood.

The generation of thermodynamically more stable enolate by treatment of the diastereoisomeric mixture with sodium hydride in tetrahydrofuran in the presence of an excess of methyl iodide, allowed stereoselective introduction of the methyl group at C2, leading to the formation of Georgywood in good yield (60%), as the only diastereoisomer, with a trans stereochemistry of the two methyl groups as demonstrated by NMR experiments.

Bella at al [107] have demonstrated the new procedure leading to the bicyclo skeleton that shortened the previously reported two step sequences. They have showen that the octahydronaphthalene skeleton can be directly and readily achieved in high yields and regioselectivity by an original tinchloride catalyzed domino process, characterized by a sequence of an intermolecular oriented Diels–Alder reaction, immediately followed by an intramolecular cyclization to give the bicyclo compound. The key-intermediate, the 2,4-dienone, gave an excellent performance in regio- and stereoselective double alkylation reactions, making the target compound easily available. Therefore, these new transformations can be efficiently utilized in target-oriented syntheses. The results show the good flexibility of a strategy, which allows preparation of a library of differently alkylsubstituted octahydronaphthalenes, valuable in the chemistry of odorants [107].

Patchouli, the name of which was borrowed from the Tamil *'patch ilai'* for *'green leaf*', became popular in Europe in the early 19th century with the fashion of cashmere shawls. To protect the fine cashmere wool on its long voyage to Europe, the precious folds of cloth were layered with leaves of patchouli, which was the most effective moth repellant then known. Its woody-balsamic scent with its well-balanced herbaceous, earthy, camphoraceous, and floral facets soon turned out to be as much a draw for buyers as the colorful cashmere itself, and thus patchouli rose from a bug repellant to a popular perfumery raw material. Kraft et al. [108] have gained additional insight in the structure–odor requirements of the patchouli receptor(s) [108].

Though the third dimension of the receptor models of *J. E. Amoore* rarely was exceeding 4 Å, the world of woody odorants such as (+)-cedrol (cedarwood), (−)-khusimone (vetiver), and (−)-patchoulol (patchouli) is anything but flat. Any tricyclic skeleton with a zero-bridge contains a spirocyclic ring system determining its 3D structure, so spirocycles are the fastest access to the third dimension. A 5-Å

distance between a quaternary C-atom and a carbonyl group (or alternative HB acceptor) with an *α*-methyl or methylene branching is proposed to be the key to their vetiver odor. Upon scale-up of one of these odorants, they have discovered a very powerful impurity with a most typical patchouli scent: the spirocyclic, sterically crowded hydroxy ketone, a most unusual structure for a patchouli odorant. Several spirocyclic hydroxy ketone analogs, also with inverted ring systems such as provided new insights into the structure–odor correlation of this family. A superposition analysis indicated the carbonyl function of the hydroxy ketone to overlay on the geminal dimethyl motive of (−)-patchoulol. Finally, the synthesis and olfactory properties of twelve rigid spirocyclic analogues of *Georgywood*® are presented that highlight stereochemical requirements for woody odorants and raise doubts about an *α*-helical binding motive postulated by Hong and Corey [108,109].

Spirocyclic analogues of Georgywood® and unlike-10-acetyl 9,10-dimethylbicyclo[6.4.0]-dodec-1(8)-ene, were synthesized. By simple LDA-mediated alkylation of the resulting spirocycles with methyl iodide, further analogues were prepared that provided additional insight into the structural and stereochemical requirements for the typical woody-ambery odor character of Iso E Super® [110].

Georgyone and arborone, powerful woody odorants, have been synthesized enantioselectively along with their enantiomers. These studies have led to a number of conclusions regarding the structural requirements for woody odor, including absolute configuration, critical methyl substitution, and the spatial orientation of the key methyl groups. Odorants bind to at least 10 mouse olfactory receptors, lending support to the combinatorial model for odor perception/differentiation. Although it may seem surprising that any individual OR can be activated by a number of ligands, it is not unreasonable that ORs which can accommodate multiple ligands would be evolutionarily favored. This result suggests the possibility that similar odorants may activate neighboring glomeruli, an intriguing aspect of olfactory organization of information. Although the biological studies are still at an early stage, it is of some interest that glomeruli that are activated by woody odorants seem to lie together as close neighbors on the olfactory bulb of the mouse, even if they are chemically and structurally different; for example, longifolene (which has a woody odor) activates some of the same glomeruli and also neighboring glomeruli as both above mentioned odorants [111].

Borosy et al. [112] have applied endo-selective Diels-Alder reaction for the synthesis of Georgywood. Diels-Alder reactions of alkyl-substituted dienes with acrylonitriles furnish good yields and endo-selectivities if catalyzed by (organo)aluminum, (organo)boron or gallium halides. This method gives the best endo/exo-ratios reported so far for these components and was applied in the selective synthesis of the olfactory vector of Georgywood® [112].

Furthermore, in this study has also the Diels-Alder reactions of ethyl α-bromoacrylate with open-chain dienes been used. In most cases, the cyclic adducts of 1-bromocyclohex-3-enecarboxylates were formed in high yields with good regio- and stereoselectivity. Subsequent E2-elimination by treatment with DBU provided the corresponding 1,3- or 1,4-cyclohexadienecarboxylates depending on the relative configuration of the products. Starting from 7-methyl-3-methyleneocta-1,6-diene (myrcene) the reaction sequence afforded the ester precursor of *Georgywood* with good yields [113].

4.3 Ambergris

Ambergris (Amber), is a solid waxy substance formed in the intestine of the sperm whale (*Physeter catodon*). In Eastern cultures ambergris is used for medicines and potions and as a spice; in the West it was used to stabilize the scent of fine perfumes. Fresh ambergris is black and soft and has a disagreeable odor. When exposed to sun, air, and seawater, it hardens and fades to a light gray or yellow, developing a subtle and pleasant fragrance in the process.

Chemically, ambergris contains alkaloids, acids, and a specific compound called ambreine, which is similar to cholesterol. Ambergris, contains about 80% cholesterol. Ambergris was commonly ground into a powder and dissolved in dilute alcohol. Rarely used today due to trade restrictions, its unique musky character added a long-lasting bouquet to the scent of essential flower oils, but, more important, ambergris was a fixative that prevented fragrance from evaporating. Some chemical components of ambergris are now produced synthetically [114].

In 2004 Kovatcheva et al. [115] have developed a combinatorial quantitative structure−activity relationships (Combi-QSAR) approach and applied it to a data set of 98 ambergris fragrance compounds with complex stereochemistry. The Combi-QSAR approach explores all possible combinations of different independent descriptor collections and various individual correlation methods to obtain statistically significant models with high internal (training set) and external (test set) accuracy. Seven different descriptor collections were generated with commercially available programs: MOE, CoMFA, CoMMA, Dragon, VolSurf, and MolconnZ; also included chirality topological descriptors recently developed [115].

CoMMA descriptors were also used in combination with MOE descriptors, further MolconnZ descriptors were used in combination with chirality descriptors. Each descriptor collection was combined individually with four correlation methods, including *k*-nearest neighbors (*k*NN) classification, Support Vector Machines (SVM), decision trees, and binary QSAR. This gave rise to 28 different types of QSAR models. Each model with high values of leave-one-out cross-validated correct classification rate for the training set was subjected to extensive internal and external validation to avoid overfitting and achieve reliable predictive power. Two validation

techniques were employed, i.e., the randomization of the target property, known as the Y-randomization test, and the assessment of external prediction accuracy using test sets. In this study the authors demonstrated that not every combination of the data modeling technique and the descriptor collection yields a validated and predictive QSAR model. *k*NN classification in combination with CoMFA descriptors was found to be the best QSAR approach [116].

Kovatcheva continued to investigate in the same area of amber odorant using an 3D QSAR procedure. Descriptors used in 3D QSAR studies take into account chirality; however, for flexible and structurally diverse molecules such studies require extensive conformational searching and alignment. The authors developed QSAR modeling studies of two datasets of fragrance compounds with complex stereochemistry using simple alignment-free chirality sensitive descriptors. 44 α-campholenic derivatives with sandalwood odor were represented as derivatives of several common structural templates with substituents numbered according to their relative spatial positions in the molecules. Both molecular and substituent descriptors were used as independent variables in MLR calculations. Further, several types of chirality descriptors were employed in combinatorial QSAR modeling of 98 ambergris fragrance compounds. Among 28 possible combinations of seven types of descriptors and four statistical modeling techniques, k nearest neighbor classification with CoMFA descriptors was initially found to generate the best models. The same dataset was then studied using novel atom pair chirality descriptors (cAP). The resulting models were found to have higher predictive power than those developed with CoMFA descriptors. The success of modeling studies using simple alignment free chirality descriptors discussed in this paper suggests that it should be applied broadly to QSAR studies of many datasets when compound stereochemistry plays an important role in defining their activity [89].

Svitanko et al. [117] published a study by using 3D-QSAR modeling that represent the electrostatic molecular surface. The method allows to take into account the spatial electrostatic complementary character of two or several molecules or a molecule and a receptor. For this method, it is necessary to calculate the electrostatic field created by the molecule and then supplement the model thus obtaind by structural complementarity. The sample consisted of 50 compounds represented by 3D molecular graphs. Geometry optimization and charge calculation were

performed by Gaussian03. For every molecule in the test set, a triangulated molecular surface with excluded solvent was constructed, using MSMS (program written in the C programming language to compute molecular surfaces). The authors picked critical points on molecular surfaces using Conollys methodology to describe local holes and knobes. At the end, simple electrostatic Coulomb potentials of every critical points were calculated by adding up the effects of electrostatic fields created by individual atoms [117].

Gorbachov et al. [118] have used a new electronic–topological approach to define an active ambergris fragment (AAF) which correctly describes the presence of the ambergris odor of all investigated 181 compounds. The AAF consists of one oxygen atom and three carbon atoms (α,β,γ) which are separated by certain key distances and which possess certain atomic charges. The C_α atom must bear at least one hydrogen atom (H_α) which is located at a certain distance from one of the unshared electronic pairs of the oxygen atom. This investigation has used the largest data set of related active and inactive compounds to date, and has included several non-*trans*-decalin examples, such as the ambergris-smelling and odorless diastereoisomers of the aroma chemical registered under the tradename of Karanal® [118].

Further development using electronic-topological approach were published 2001 by Dimoglo et al. [119]. They have investigated a series of 201 compounds with decalin- and non-decalin-type skeletons regarding the possession of ambergris odor. A structural fragment of activity (FA2) has been identified, which is a refinement of an activity feature (FA1) obtained as a result of an earlier electronic-topological study of the structure–odor relationship in a series of decalin-type compounds. The FA2 fragment consists of an oxygen atom and five carbon atoms situated in both decalin and cyclohexane parts of the molecules and possesses well-defined three-dimensional (3D) topology and strictly defined electronic characteristics [119].

Studies of Cheng et al. [120], are based on quantum chemical calculation method. Structural and electronic origin study shows the correlation between structural, stereochemical as well as electronic features and ambergris odor of some tricyclic ethers, established based on quantum chemical calculation method. The influence of HOMO-LUMO energy gaps and total energies of some ambergris compounds on their odor intensity is investigated. A definite structural fragment of an new

ambergris odor with certain electronic properties determining the origin of the odor is revealed [120].

Ten new derivatives of ambrein, isolated from ambergris, were prepared by chemical transformation by Shen et al. [121]. The structures of new derivates were elucidated by spectroscopic analysis, using single-crystal X-ray crystallography. The cytotoxic activities of new derivates were investigated against human liver carcinoma, colon adenocarcinoma, lung carcinoma, and human breast adenocarcinoma cell lines. The anti-inflammatory activities of many compounds, in terms of the inhibition of human neutrophil function, were also evaluated [121].

One of the most important ambra odorants is Ambrox. Today it is synthesized from the diterpene sclareol, that was found in the plant Clary Sage. Ambrox of high quality is marketed as Cetalox® by Firmenich and as Ambrofix® by Givaudan. Winter [122] has published a study about synthesis and structure-odor relationships of spirocyclic ethers related to *Ambrox.* Seven spirocyclic ethers, related to the tricyclic odorant Ambrox and its diastereoisomers, were synthesized. Their odoriferous activity/inactivity was correlated with the steric accessibility of the ether O-atom, calculated by computer-aided molecular modeling. These results underline the inherent premise that the easily calculated steric accessibility of the functional group(s) in any bioactive compound is only one parameter among many [122].

4.4 Musk

Musk odorous substance secreted by an abdominal gland of the musk deer, used in perfume as a scent and fixative. The gland, found only in males, grows to the size of a hen's egg. The secretion is reddish-brown, with a honeylike consistency and a strong odor that may function in the animal as a sexual attractant. After the pouch is cut the secretion hardens, assumes a blackish-brown color, and when dry becomes granular. Usually a tincture of alcohol is made from the grains, which will be added to expensive perfumes. The chief constituent that gives musk its odor is the organic compound *muscone*. Musklike substances are also obtained from the muskrat and the civet. Some plants such as *Angelica archangelica* or *Abelmoschus moschatus* produce musky smelling macrocyclic lactone compounds. Some plants yield oils which resemble musk; these include the seed of ambrette (*Hibiscus abelmoschos*) and the sumbul root (*Ferula sumbul*) of central Asia and Turkistan. A number of synthetic musklike products are also used now. Until the late 19th century, natural musk was used extensively in perfumery until economic and ethical motives led to the adoption of synthetic musk, which is used almost exclusively. These compounds are widely used in perfumery as substitutes for animal musk or to alter the smell of a mixture of other musks [123].

Musk odorants have been a classical domain for computer aided structure-odor relationship studies, but contrary to sandalwoods or amber odorants they belong to three structurally very different substance classes: macrocycles (*Exaltolide, Thibetolide*), aromatic polycycles (*Phantolide , Galaxolide*) and nitro arenes (such as Musk ketone). Most SOR computer models are restricted to one class, excluding structural diversity to increase predictability. Within musk family, structural similarities are often due to a common synthetic access and do not reflect binding requirements for the musk receptor [124, 125].

Among the three well known classes of musks, Eh [126] discoverd a new generation of musk odorants, so called alicyclic musks, of witch *Helvetolide* and *Romandolide* are the most popular representatives so far. They have established a solution-phase parallel synthesis of a large number of new alicyclic musk odorants. The advantage

of this approach is that it allows the rapid preparation of various analogs for the study of structure-odor relationships. The syntheses and olfactory revolutions of eight new macrocyclic musks with a 1,6-dioxa structure as well of twelve opticaly active 3-methyl macrolides are reported. Despite of absence of a C=O function, the 1,6-dioxa compounds possess musky odors. Especially 16-membered rings were found to display an intense and pleasant character [126].

Structure-odor relationship analyses using hierarchical clustering were used on a dataset of 47 molecules. These molecules were divided into seven odor categories: ambergris, bitter almond, camphoraceous, rose, jasmine, muguet, and musk. The dataset of the musk molecules contains five macrocyclic musks, four nitro musks, and two non-nitro aromatic benzenoids. The alignment-independent descriptor EVA (EigenVAlue) was used as the molecular descriptor. The results were compared with those of another descriptor, the UNITY 2D fingerprint. The dendrograms obtained with these descriptors were compared with the seven odor categories using the adjusted Rand index. The dendrograms produced by EVA consistently outperformed those from UNITY 2D in reproducing the experimental odor classifications of these 47 molecules. Inspection suggests structural similarity between the ambergris molecules and the benzenoid musks in a dataset [127].

In a rewiev Frater et al. [128] illustrated what chiral recognition tells us about the molecular parameters of the musk odor sensation. While the enantioselectivity of odor perception is strong evidence for the key role of proteinogenic receptors in the molecular mechanism of olfaction, the quantitative and qualitative odor differences of enantiomers are often not very pronounced, as in the case of muscone. The authors have found most intense musk odorants with very low odor thresholds, such as (-)-(12*R*)-12-methyl-9-oxa-14-tetradecanolide, (12*R*;9*Z*)-12-methyl-14-tetradec-9-enolide [(*R*)-*Nirvanolide*], and (-)-(4*S*;7*R*)-1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran [(-)-(4*S*;7*R*)-*Galaxolide*]. Thus can assume the geometry of the musk receptor to be fairly complementary to these compounds, which therefore can serve as templates for the design of new musk odorants [128].

Four new representatives of the dienone musks were synthesized. The four target compounds were designed as diseco derivatives of a carotol lead, and they all constitute musk odorants with floral-fruity side notes. A tert-butyl group at the C-6 position of the (*E*)-hexa-3,5-dien-2-one skeleton was found to intensify the musk

odor, and (*E*)-4-(2'-tern-butyl-5',5'-dimethylcyclopent-1'-enyl)but-3-en-2-one was found the most intense and interesting odorant of the series, with a very uncommon undertone of beetroot and dried fruits. In a new study the synthesis of several new acetylpolyalkylindans, musk odorants, have been reported [129]. The hexamethyl compound is the most important of these and has the best properties as a musk substitute [129].

4.5 Other Odors

Oils from *Angelica Archangelica* L. are important ingredients in flavor formulations used in alcoholic-beverage industry and in lower dosages also in preparation of fine fragrances. The seed oil is a light yellow liquid with a fresh, sweet and peppery odor. The root oil is a pale yellow to deep amber liquid with a green, herbaceous, peppery musk-like odor and bittersweet taste. The most valuable root oils are comprised of unique musk-like compounds.

The chemical composition of seed and roots oil of *Angelica Archangelica* L. was studied in detail. The result was identification of 14-methylpentadecano-15-lactone in a neutral product. Analyses were performed by GC/MS and GC. A total of 58 compounds were identified by Lopes et al. [130]. A high content of β-phellandrene was found in Angelica seed oil [130].

Honey is produced by honeybees (*Apis mellifera*), which collect nectar from flowers, digest it in their bodies and deposite it in honeycombs, where it develops into ripe honey. In a study of Naef et al. [106] the evolution of volative constituents from the nectar of Linden blossoms (*Tilia cordata*) has been reported. Extracts were prepared from nectar, from the liquid of the honey stomach and from ripe honey. It showes extremly complex chemistry of compound spanning from monoterpenes, isoprenoids, aromatic compounds and products degraded from fatty acids to alkaloids. Their observations are based on the interpretation of restricted samples of a complex sequence of event. This report ist a subjective selection of results that seem to delineate the evolution of the volatile costituents from the flower nectar to honey [131].

Previous studies have attempted to describe olfactory generalisation in honeybees and to study structure–activity relationships. These studies generally supported the view that generalisation mainly happens when odors belong to the same chemical group. Furthermore, these studies were carried out on a rather limited number of odor pairs, did not detail the results obtained with individual odor combinations or used a very reduced number of bees per conditioned odor (two bees per odorant). The present study by Guerrieri et al. [132] is the first one to provide generalisation data based on absolute conditioning, a systematic test of all odor combinations,

robust sample sizes for each experimental situation, and important generalisation gradients. Guerrieri et al. [132] have studied the olfaction in honeybee *Apis mellifera* using the olfactory conditioning of the proboscis extension response. The authors conditioned bees to odors and tested generalisation responses to different odors. Sixteen odors were used, which varied both in their functional group (primary and secondary alcohols, aldehydes and ketones) and in their carbon-chain length (from six to nine carbons). It was shown that the two odorant physical dimensions that varied in the study, functional group and chain-length, correspond to internal dimensions of the bees' olfactory space. Generalisation was mainly due to these two characteristics with generalisation within functional group being more important.
It was concluded that functional group and carbon-chain length are inner dimensions of the honeybee olfactory space and that neural activity in the antennal lobe reflects the perceptual quality of odors. The work shows that this objective, which is at the core of cognitive neurosciences, can be achieved using an invertebrate model such as the honeybee [132].

Violet smelling ionones, occurring in the headspace of different flowers, are well known perfumery raw materials. With the goal to recognize the still ill-defined spatial arrangement of structural features relevant to the binding of ionones to olfactory G-protein coupled receptors, through B3LYP/6-31G modeling studies Luparia et al. [133] have identified bicyclic compounds as conformationally constrained 13-alkyl-substituted analogues of monocyclic α- and γ-ionones. These were synthesized to evaluate the olfactory properties. Modeling studies suggested a nearly identical spatial orientation of key hydrophobic and polar moieties of compounds. Presumably, interaction of these moieties with ionone olfactory receptors triggers a similar receptor code that is ultimately interpreted by the human brain as a pleasant woody-violet smell [133].
Jasmal® and Jessemal® are odorants commercialized by many companies under different trademarks. These fragrances are used in many types of cosmetic products, and both have a pleasant odor of jasmine flower. Abate et al. [134] reported on the preparation and the olfactory description of four possible stereoisomers of the fragrance Jasmal® and four of the eight possible stereoisomers of the fragrance Jessemal®. The olfactory evaluations have shown that the absolute configuration of

odorant molecules plays an important role in the human perception of odor. Concerning the synthesis of the four stereoisomers of the Jessemal®, the authors wanted to mention that the preparation of an optically pure compound by means of an enzymatic resolution of the racemic alcohol, represents an efficient and simple synthetic alternative to the other reported methods, which are all based on the epimerizable aldehyde [134].

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II. Teil

1. EINLEITUNG UND FRAGESTELLUNG

" Ein Raum ohne Buch ist wie ein Körper ohne Seele", schrieb bekanntlich Cicero.
Ziel dieser Arbeit war den Geruch, die Duftstoffe, die im Antiquariat vorkommen, zu untersuchen. Ein Antiquariat ist ein auf alte und gebrauchte Bücher spezialisiertes Geschäft**.** Heutzutage gibt es auch moderne Antiquariate, die neben alten und gebrauchten Büchern auch neue Auflagen verkaufen.
Alte Bücher zeigen einen charakteristischen Geruch, welche im Antiquariat Schaden, ein Familienbetrieb mit einer 50-jährigen Tradition als Buchhandlung und Antiquariat, untersucht wurde. Für diesen typisch staubigen, muffigen, schimmeligen und papier-ähnlichen Geruch sind auch die Vielzahl von Materialien, die für Buch Produktion verwendet werden (Papier, Tinte, Klebstoff...), mitverantwortlich. Zum Geruch tragen auch andere Substanzen, die von Möbeln (Holz, Lack...), Reinigungsmitteln, Parfümkomponenten und anderen Quellen, welche typisch für Innenräume sind, bei.
Diese Messungen fanden im Rahmen eines Projekts des Wiener Wissenschafts-, Forschungs- und Technologiefonds (WWTF)– "*Haptic and Olfactory Design, Resources for Vienna's Creative Industries"* statt**.** Dabei wurden wissenschaftliche Untersuchengen von für Wien typischen Plätzen und Einrichtungen durchgeführt.
Um bestmögliche Ergebnisse der Antiquariat-Analysen erzielen zu können, wurden im Vorfeld einige Versuche getätigt, welche die nachfolgenden Resultate optimieren sollen. Hierzu führte man die ersten Raumluftmessungen in der Universitätsbibliothek durch. Eine weitere vergleichende Analyse erfolgte an einem alten Buch, welches in geschützter Atmosphäre eingelagert und so mittels der Solid-Phase Micro-Extraction, „SPME" auf dessen "Eigengeruch" analysiert wurde.
Dies soll die Detektion und die Zuordnung von Substanzen erleichtern**.** Beide Vortests sind für die weiteren Analysen der Innenraum-Luft des Antiquariats sehr hilfreich, da sie aufgrund derselben Methodik im GC-MS zum Vergleich herangezogen werden können.
Zur Probensammlung wurde das Antiquariat ``Schaden``, 1010 Wien, Lugeck 7/2, ausgesucht. Es ist ein kleines Geschäft im Zentrum von Wien, das sowohl alte als auch neue Bücher anbietet.

Die Proben wurden mittels SPME im Zeitraum von vier und sechs Stunden genommen. Es wurden immer zwei Sampler verwendet, um eine Doppelbestimmung durchzuführen. Eine SPME-Faser wird in Raum orientiert und die andere mehr in Richtung Bücher. Damit wurde die Umgebungsluft gesammelt, um so auf die Spur des typischen Duftes des Antiquariats zu kommen.

Mittels GC-MS-Technik konnten die einzelnen Riechstoffe getrennt und analysiert werden. Dabei dient der Gaschromatograph zur Auftrennung des zu untersuchenden Stoffgemisches in seine Einzelkomponenten und das Massenspektrometer zur Identifizierung der getrennten Komponenten. Es wurde mit einer Methode gearbeitet, welche sich bei früheren Diplomarbeiten, welche z.B. die Innenraumluft von Kaffeehäusern analysierten, als erfolgreicht erwiesen hat.

Das Massenspektrometer identifiziert jede einzelne Substanz. Auf diese Weise erhält man ein Spektrogramm, anhand dessen Aroma- und Innenluft-Bestandteile genau bestimmt werden. Die Auswertung und Interpretation der Spektren erfolgt durch Vergleich von gemessenen Spektren mit den Spektren aus handelsüblichen Bibliotheken. Die Datenerfassung und Auswertung der getätigten Messungen erfolgte durch die Software *Xcalibur* des GC-MS-Gerätes (Thermo Fisher Scientific Inc.). Die erhaltenen Massenspektren wurden anhand folgender Spektrenbibliotheken ausgewertet:

- Wiley Registry ^{TM} of Mass Spectral Data 8th Edition
- NIST/EPA/NIH Mass Spectral Library 1.5a (match factors >850).

Es ist signifikant, dass sich die Chromatogramme nur in den Konzentrationen der einzelnen Komponenten, nicht aber drastisch in ihrer Gesamtzusammensetzung, unterscheiden.

In den Chromatogrammen wurden flüchtige Abbauprodukte mit wichtigen Eigenschaften für die Erhaltung von historischem Papier: Harz, Lignin und Carbonylgruppen, mittlere und höhere Aldehyde und Alkylcarbonsäuren gefunden. Außerdem wurden Substanzen ermittelt, die von Reinigungsmitteln, Parfüms und/ oder von Speisen stammen.

2. LITERATURÜBERSICHT

2.1.Antiquariat-Definition

Das moderne Lexikon definiert den Begriff Antiquariat als: Handel mit alten Büchern, alt im doppelten Sinne; mit Büchern aus früheren Jahrhunderten (insbes. etwa Frühdrucken), oder mit solchen, die neueren Datums, aber gebraucht sind, schließlich mit solchen, für die der Verlag – soweit Preisbindung besteht – den Ladenpreis aufgehoben hat.

Die Bezeichnung gelangte seit dem 18. Jahrhundert, zuerst in England und Frankreich, zur Anwendung. Das Wort stammt vom lat. „Antiquarius“ [1].

Abbildung 2 Bücher im Antiquariat
Foto : Koscak, 2009

Als Antiquariat wird ein Geschäft bezeichnet, in dem man alte und gebrauchte Bücher oder andere alte Schriften wie Handschriften, Autographen, Zeitungen, Land- oder Seekarten, Graphiken kaufen kann. Das Fachpersonal wird hierbei nicht Buchhändler, sondern Antiquar genannt. Er ist spezialisiert darauf, seltene und nicht mehr erhältliche Bücher aufzustöbern, zu erwerben, zu verwahren und zu verkaufen. Dabei ist es wichtig, den angemessenen Preis und damit den Wert des antiquarischen Buches zu ermitteln. Außerdem sorgt der Antiquar natürlich auch für eine schützende Aufbewahrung der älteren Werke.

Ein Antiquariat ist demnach die erste Anlaufstelle, wenn man auf der Suche nach einem längst vergriffenen, seltenen Buch ist. Als Kunde findet man in einem Antiquariat oft jene alten Bücher, die man möglicherweise schon lange gesucht hat [2].

2.2 Geschichte des Antiquariates

Das Antiquariat, dem man Heute kennt, gibt es erst seit etwas mehr als 200 Jahren. Das Antiquariat bildete sich seit dem Beginn des 17. Jahrhunderts als Teil des Trödelhandels und Mitte des 18. Jahrhunderts mit der Trennung von Verlag und Sortiment heraus [3].

Ende des 17. Jahrhunderts gab es in Wien, der Stadt von nur etwas über hunderttausend Einwohnern, acht Buchdrucker und sechs Buchhandlungen. Antiquariatshandel wurde von 1772 von Buchhändelerordnung als fix vorgesehen [4].

Wiener Antiquariate wurden schon im 17. Jahrhundert von einigen Autoren erwähnt. Eine der bedeutensten ist Johann Pezzl, welcher am Ende der 1780er Jahre einige „Bücherantiquare“ zur Zeit des Josephinismus erwähnte. Als weiteres zählt Franz Gräffer 1835 in der „Österreichischen National-Enzyklopädie“ einige bedeutende Antiquariate der Monarchie auf; dessen Ausführungen werden später von Carl Junker übernommen [5].

2.3 Probenanreicherung– Solid Phase Microextraction (SPME, Festphasenmikroextraktion)

Festphasenmikroextraktion (englisch *solid phase microextraction*, *SPME*) ist eine einfache und leistungsfähige Extraktionsmethode, die von Pawliszyn 1990 entwickelt wurde. Bei den SPME handelt es sich um eine Methode der Probenahme und Analytenanreicherung, welche sich in einigen Bereichen der chemischen Analytik als vorteilhaft gegenüber klassischen Methoden wie z. B. "Purge and Trap" oder SPE (Solid Phase Extraction, Festphasenextraktion) erwiesen hat. Die Solid Phase Micro Extraction SPME eignet sich hervorragend zur Anreicherung organischer, mehr oder weniger flüchtiger Verbindungen aus wässrigen Medien oder aus dem Dampfraum über der Probe (Headspace). Dabei kommt eine mit einem Adsorbens beschichtete SPME-Faser zum Einsatz, die entweder in eine Flüssigkeit getaucht oder im Headspace positioniert wird.
Der alleinige Lizenznehmer Weltweit für Festphasenmikroextraktion-Technologie ist Supelco®.
Die Methode hat viele Vorteile gegenüber klassischen Methoden:

- kein Lösungsmittel erforderlich (erhebliche ökologische wie ökonomische Vorteile)
- keine schwierige Apparatur
- Verzicht auf aufwändige Probenvorbereitung, bedeutet, dass die Proben weniger verändert werden
- Es gibt die Möglichkeit der Automatisierung
- Bei sorgfältiger Behandlung kann eine Faser ohne Probleme für wenigstens 50 Analysen verwendet werden. Nach jeder Extraktion muss die SPME-Faser von Analytrückständen im heißen Injektor Block bei 230 bis 250°C für 10 Minuten gereinigt werden.

Dadurch zeichnet sich SPME sowohl durch eine schnelle Durchführung sowie geringe Kosten in Anschaffung und Anwendung aus. Die SPME etabliert sich immer

stärker als prä-analytische Methode vor allem innerhalb der Probennahme von Analyten in komplex zusammengesetzten industriellen oder biologischen Matrices.
Die Festphasenmikroextraktion ist sowohl eine Art Probenvorbereitung als auch eine Injektionstechnik, hauptsächlich benutzt in der GC, aber auch anwendbar in der HPLC [6].

Die Probennahme erfolgt hauptsächlich durch Adsorption (im geringen Maβ auch durch Lösen in der Schicht). Anreicherung der Analyten an eine mit Polymer beschichten *Fused-Silica* Faser (Extraktion), anschließende Desorption im Gaschromatographen (GC) und Gaschromatographie in Kopplung mit Massenspektrometrie (GC-MS) oder Hochleistungs-Flüssig-Chromatographie (HPLC) [7].

Die Anwendungsmöglichkeiten der SPME sind vielseitig, vor allem durch nachfolgende Kombination mit einer gaschromatographischen Analyse.
Die Festphasenmikroextraktion wird im Bereich

- Umweltanalytik (z. B. Bestimmung von Herbiziden im Trinkwasser Pestizidnachweiss)
- Lebensmittelanalytik (z. B. Bestimmung von durch Licht erzeugten Abbauprodukten in Milch)
- Aromaanalytik (z. B. Bestimmung der Geruchsstoffe in Blütendüften)
- Forensik (z. B. Bestimmung von VOCs, Volatile Organic Chemical, im Urin)
- Pharmazeutischen Industrie
- Probenaufgabe in der Gaschromatographie (spezielle Headspace-Technik) [6].

Die *Nachweisgrenze* bewegt sich zwischen 0.01 ng/l (ppt) und 9 mg/l (ppm) –dieser weiter Konzentrationsbereich ist vor allem auf die unterschiedlich eingesetzten Detektoren zurückzuführen [8].

Aufbau der SPME :

Die SPME-Probennehmer ist ein sehr einfaches Gerät, das einer Injektionsspritze ähnelt.

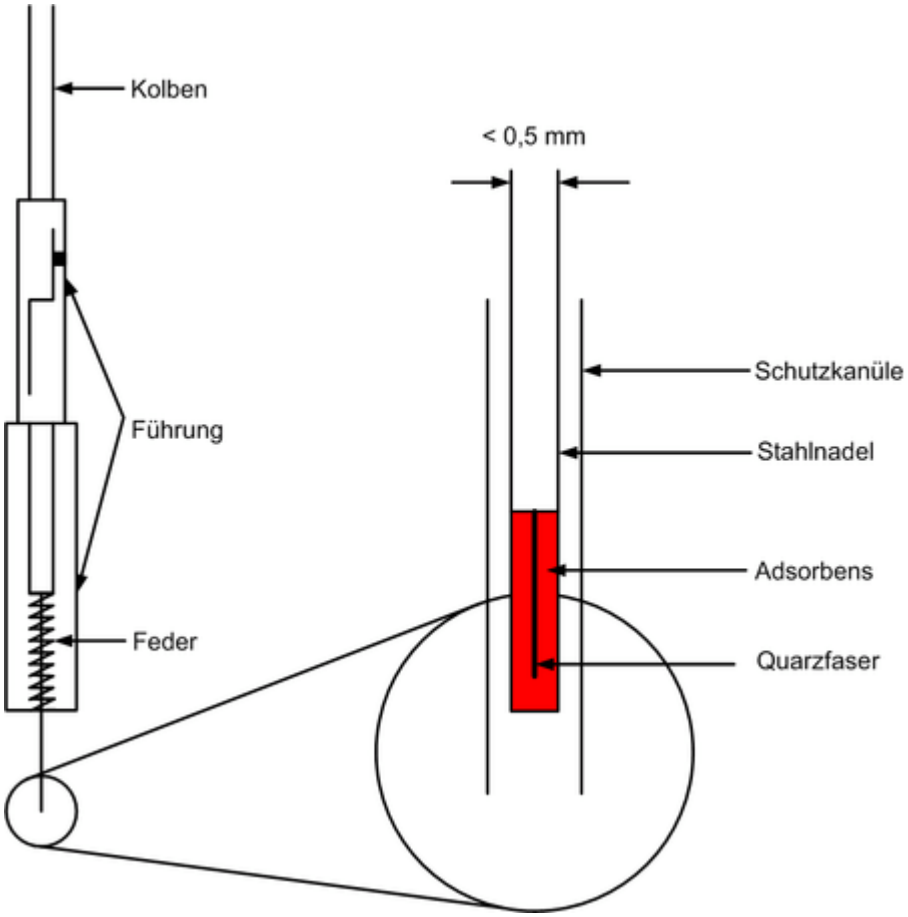

Abbildung 3 Schematische Darstellung: SPME-Faser und Faserhalter
Quelle: *[STIEN, 2001]*

Der Probennehmer besteht aus:

- Einer Führung für einen Kolben. Die Führung besitzt einen Bajonett-Verschluss, um den Kolben im niedergedrückten Zustand arretieren zu können.

- Einem Kolben zum Ausfahren des Probenehmers. Am Ende des Kolbens befindet sich eine Feder, die den Kolben wieder zurückschiebt, wenn die Arretierung gelöst wird.
- Dem eigentlichen, an den Kolben angeschraubten Probenehmer- *Kernstück* der Festphasenmikroextraktion. Er besteht aus einer Edelstahlnadel die während der Adsorption/Desorption heraus bzw. hinein geschoben werden kann. An der Nadel befindet sich eine 1-2 cm lange Quarzfaser (Fused Silica Faser). Diese Quarzfaser ist mit einer dünnen 5 bis 100 µm starken Schicht Adsorbens überzogen. Die Beschichtung der Faser gibt es in verschiedenen Polaritäten und Selektivitäten: Polyacrylat, Carbowax, Polydivinylbenzen, Carboxen und das meist verwendete PDMS, Polydimethylsiloxan.
- Einer Schutzkanüle, durch die die Faser aufgrund ihrer geringen mechanischen Stabilität geführt wird [9].

Prinzip der SPME

An der Oberfläche der SPME-Faser, einer dünnen, geschlossenen Kapillare, die mit einer stationären Phase beschichtet ist, werden die Analyten zunächst adsorbiert (und angereichert). Die anschließende Desorption erfolgt thermisch im Injektor des Gaschromatographen oder im Injektor der HPLC durch den Eluenten.
Man kann die SPME einerseits als Headspace-Methode durchführen, d.h. wenn die Analyten ausreichend flüchtig sind, erwärmt man die Probe in einem mit Septum verschlossenen Gefäß unter Rühren und hält die Faser dabei in den Dampfraum. Die flüchtigen Substanzen reichern sich im Dampfraum an und werden an der Faseroberfläche adsorbiert. Andererseits kann man bei schwerer flüchtigen Substanzen die Faser direkt in die flüssige Probe tauchen. Unter Rühren werden die Analyten an der Faser adsorbiert und angereichert [8, 6].

2.4. Grundlagen der Gaschromatographie (GC)

GC ist ein physikalisch-chemisches Analyseverfahren zur qualitativen und auch quantitativen Bestimmung der Inhaltsstoffe von Gasgemischen. Die Gaschromatographie ist eine spezielle Methode innerhalb der Chromatographie, es handelt sich hauptsächlich um Verteilungschromatographie, bei der die mobile Phase aus dem im trennenden Gasgemisch besteht. Dieses Gas wird durch eine Säule geleitet, die mit bestimmten Materialien ausgekleidet (= stationäre Phase) ist. Das Trägergas, das in der Gaschromatographie verwendet wird, muss inert sein. Im Normalfall wird Stickstoff, Argon oder Helium verwendet.

Das Material der Säulen besteht entweder aus Metall oder aus mit Polyamid beschichtetem Quarzglas (= Kapillarsäulen). Die zu untersuchenden Substanzen erreichen nacheinander das Säulenende und werden durch einen Detektor mit Hilfe einer Auswerteeinheit als Peaks angezeigt. Die qualitative Auswertung erfolgt über die Retentionszeit, die quantitative Auswertung geschieht über die Flächenermittlung durch Integration. Grundbedingung in der Gaschromatographie ist, dass sich die Substanz, die man untersuchen möchte, unzersetzt verdampfen lässt, sofern sie nicht schon gasförmig vorliegt [10].

2.5. Grundlagen der Massenspetrometrie (MS)

Die Massenspetrometrie ist eine Methode der instrumentellen Analytik, bei der mit kleinsten Substanzmengen die relative Molekülmasse und sogar die Elementarzusammmensetzung einer Verbindung bestimmt werden kann. Wichtige Aussagen über die Struktur sind durch das Zerfallsmuster des Materials unter Elektronenbeschuss möglich. Bei Massenspektrometrie werden aus dem Analyt Ionen erzeugt, diese entsprechend ihres Masse/Ladungs-Verhältnisses (m/z) in einem Analysator aufgetrennt und anschließend registriert. Als Ergebnis erhält man ein Massenspektrum. Das Massenspektrum wird üblicherweise als Strichspektrum dargestellt, wobei die einzelnen Peaks jeweils auf ganzzahligen Massen aufgetragen werden. Aus den Massenspektren lassen sich sowohl qualitative Informationen (über das Fragmentierungsmuster und den Molekülpeak) als auch quantitative Informationen (über den Gesamtionenstrom) erhalten.

Massenspektrometer kann als eigenständiges Gerät oder auch gekoppelt mit der Gas- oder Flüssigkeits-Chromatographie eingesetzt werden [11].

3. MATERIAL UND METHODEN

3.1. Methodenentwicklung für GC-MS-Untersuchungen mittels HS-SPME

Um bestmögliche Ergebnisse der Antiquariat-Analysen erzielen zu können, wurden im Vorfeld einige Versuche getätigt, welche die nachfolgenden Resultate optimieren sollen. Hierzu führte man die ersten Raumluftmessungen in der Universitätsbibliothek durch. Eine weitere vergleichende Analyse erfolgte an einem alten Buch welches in geschützter Atmosphäre eingelagert und so mittels der SPME auf dessen "Eigengeruch" analysiert wurde.

Abbildung 4 Buch im Exicator
Photo: Koscak,2009.

Dies soll die Detektion und die Zuordnung von Substanzen erleichtern. Beide Vortests sind für die weiteren Analysen der Innenraum-Luft des Antiquariats sehr hilfreich, da sie aufgrund derselben Methodik am GC-MS zum Vergleich herangezogen werden können .

Um bestmögliche Resultate zu erzielen, wurden Messungen von unterschiedlicher Dauer durchgeführt. Anfangs wurde das SPME-Gerät für zwei Stunden aufgestellt, was sich als zu kurz erwies. Einige Komponenten kommen im Luftraum in sehr niedrigen Konzentrationen vor, was mit kurzen Messzeiten nicht erfasst werden konnte.

Durch weitere Probeversuche hat sich ergeben, dass die Messungen von vier und sechs Stunden das beste Ergebnis zeigten.

Zur Probensammlung wurde das Antiquariat ``Schaden``, ausgesucht. Die Proben wurden mittels SPME im Zeitraum von vier und sechs Stunden genommen. Eine SPME-Faser wird in Raum orientiert und die andere mehr in Richtung Bücher.

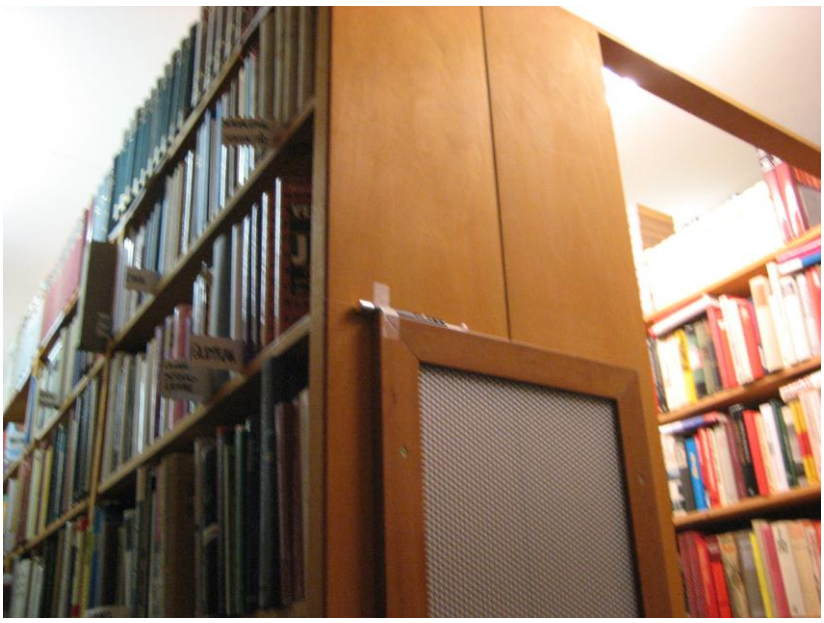

Abbildung 5 Raumluftmessengen mit SPME-Gäret im Antiquariat Schaden
Foto : Koscak, 2009

Anhand der erhaltenen Chromatogramme konnten Aroma- und Innenluft-Bestandteile genau aufgeschlüsselt werden. Die Auswertung und Interpretation der Spektren erfolgt durch Vergleich von gemessenen Spektren mit den Spektren aus handelsüblichen Bibliotheken. Da die Retenzionszeiten (Rt) für einzelne Substanzen charakteristische Größen sind, konnte man wir mittels Gaschromatographie qualitative Aussagen den zu untersuchenden Komponenten erzielen.

3.2. Entwickelte Methoden

Die Methode, die bei den Analyse verwendet wurde, hat sich bei früheren Diplomarbeiten als erfolgreich erwiesen [12].

SPME Faser

Für alle in dieser Arbeit durchgeführter Messungen wurde die für manuelle Probennahme vorgesehene SPME-Faser (57348-U, Fa. *Supelco*) verwendet. Die Faser ist im Faserhalter (57330-U, Fa. *Supelco*) verankert und dadurch geschützt.

Für diese Messungen wurde die Faser mit einer Divinylbenzen/Carboxen/Polydimethylsiloxan (DVB/CAR/PDMS)-Beschichtung der Stärke 50/30 µm verwendet. Die Faserstärke und Beschichtung hat sich schon in einigen Aromastudien bewärt [13].

Abbildung 6 SPME-Fasser
Foto: Koscak 2009

Gaschromatographische – massenspetrometrische Parameter

Die aufgetrennten Substanzen können direkt vom GC über ein Intervall in das MS gelangen, weil das Ende der Trennsäule bis in die Ionenquelle des Massenspektrometers hineinzieht.

Nach erfolgreicher Probennahme wird die SPME-Faser mithilfe eines spezifischen Faserhalters in den Injektorblock des GC eingeführt. Die Desorption der Analyten erfolgt ohne weitere Probenbereitung direkt im Injektor des Gaschromatographen. Die angereicherte Faser wird dabei in den beheizten Einspritzblock gespritzt. Die thermische Desorption wird bei 250°C für 2 Minuten im Injektorblock durchgeführt.

Für die Analyse wurden der Gaschromatograph *Trace GC Ultra* mit einer 60 m Kapillarsäule (TRACE TR-5MS GC Column, 60m x 0.25mmlD x 0.25 µm Film) der Firma *Supelco* und ein massenselektiver Detektor *DSQ II* der Firma *Thermo Fisher Scientific* verwendet.

Die Säulentemperatur wurde am Anfang für zwei Minuten auf 40°C gehalten, dann steigt die Temperatur pro Minute um 3°C bis sie die 250°C ereicht. Die Temperatur der Ionenquelle im MS betrug 200°C. Die Dauer der entwickelten Methode belief sich vom Einspritzen der SPME-Faser in den Injektorblock auf etwa 55 Minuten [12].

Abbildung 7 Gaschromatograph- Massenspektrometer, verwendet für die Analyse Photo: Pauzenberger [12].

Analytische Bedingungen

Festphasenmikroextraktion:

Probenaufgabe: manuelle HS-SPME-Vorrichtung, (Artikelnr. 57348-U, 57330-U, *Supelco*)

Fasermaterial: DVB/CAR/PDMS

Konditionierung: 20 min, 250 °C, vor Analyse

Desorption: GC-Injektor

Gaschromatographie:

Gerat: Thermo Sci., Trace GC Ultra

Trägergas: UN1046 Helium, verdichtet

Trägergeschwindigkeit: 1ml/min, constant flow

Trennsäule: 60 m (Artikelnr.: 260F154P, TR-5MS)

Innendurchmesser: 0.25 mm

Filmdicke: 0.25 µm

Injektorblock: split/splitless Injektor, Temperatur 250°C,

ausgestattet mit einem Inlet Liner für SPME,

0.75 mm lD (Artikelnr. 45352083, *Supelco*)

Injektorprogramm:

Betriebsart: splitlos (2 min)

Equilibration time: 30 Sekunden

Ofenprogramm:

Anfangstemperatur: 40°C

Haltezeit: 2 min

Heizrate: 3°C/min

Endtemperatur: 250°C

Gesamtzeit: 55.33 min

Massenspektrometrie:

Gerät: Thermo DSQ II, direkte Kopplung (Interface)

Transferline-Temperatur: 250°C

Ionenquelle: 200°C, EI

Scanbereich: 50 – 350 amu/ 0.35 sec; full scan; TIC

Elektronenenergie: 70 eV [12].

3.3. Auswertung des Massenspektrogramms

Mit Hilfe des Massenspektrometers ist es möglich jede einzelne Substanz zu identifizieren. Man erhält ein Chromatogramm, anhand dessen Aroma- und Innenluft- Bestandteile genau aufgeschlüsselt werden.

Das für die Auswertung benötigte Masse-Ladungs-Verhältnis (m/z) und Intensität der einzelnen Fragmente sind direkt aus dem Linienspektrum entnehmbar. Auf der Ordinate des Chromatograms wird die relative Intensität der Ionen dargestellt, auf der Abszisse das Masse/Ladungsverhältnis.

Beim eingesetzten EI-Massenspektrum kommen nur einfach positive Ionen vor, hier ist dann die waagerechte Achse tatsächlich die molare Masse.

Den intensivsten Peak des Massenspektrometers bezeichnet man als Basispeak, dessen Höhe wird willkürlich mit 100 angesetzt, alle anderen Peaks werden relativ dazu dargestellt, im Prozent davon angegeben. Dadurch erreicht man eine gut vergleichbare Darstellung der Spektren.

Ein weiterer wichtiger Peak ist der Molekülpeak, der das Signal bei der höchsten Masse stellt und der dem Molekulargewicht der Substanz entspricht. Allerdings ist der Molekülpeak nicht bei allen Substanzen sichtbar [10,13].

Auswertungen der GC-Chromatogramme

Die Auswertung und Interpretation der Spektren erfolgte durch Vergleich von gemessenen Spektren mit den Spektren aus handelsüblichen Bibliotheken. Dabei wird angegeben, wie gut das gemessene Spektrum mit dem Bibliothekseintrag übereinstimmt [15].

Die Datenerfassung und Auswertung der Messungen erfolgte durch die Software *Xcalibur* des GC-MS-Gerätes (Thermo Fisher Scientific Inc.). Die erhaltenen Massenspektren wurden anhand folgender Spektrenbibliotheken ausgewertet:

- Wiley Registry ^{TM} of Mass Spectral Data 8th Edition
- NIST/EPA/NIH Mass Spectral Library 1.5a (match factors >850).

Man kann die beiden Spektrenbibliotheken in Kombination anwenden oder einzeln nutzen.

Die Bibliotheken geben mehrere nützliche Daten, wie Molgewicht, Struktur, CAS-Nummer und Synonyme der zu findenden Substanzen, welcher die Auffinden der einzelnen Komponenten erleichtern.

4. ERGEBNISSE UND DISKUSSION

Die folgenden Chromatogramme in dieser Arbeit wurden anhand der Spektrenbibliotheken von WILEY und NIST ausgewertet und mithilfe von mehreren Studien, die sich mit demselben Thema beschäftigen, verglichen.
Die Messung mittels GC-MS fand im *full scan*-Modus statt. Bei Chromatogrammen handelt es sich um Totalionenchromatogramme (TIC; total ion current), die eine rein qualitative Analyse erlauben

4.1. Untersuchung eines isolierten Buches mittels SPME-GC-MS

Zur Anfang der Analyse sollte versucht werden, den Geruch eines alten Buches zu erfassen. Deswegen wurde ein Buch aus der Fachbibliothek Pharmazie der Universität Wien entlehnt und in einem Exsikkator über Nacht eingeschlossen.
Im Chromatogramm wurden mehr als 200 Substanzen detektiert. Die zahlreichste Verbindungsklasse stellten flüchtige organische Verbindungen wie Aldehyde, Ketone dar, weiters Säuren und Phenole.

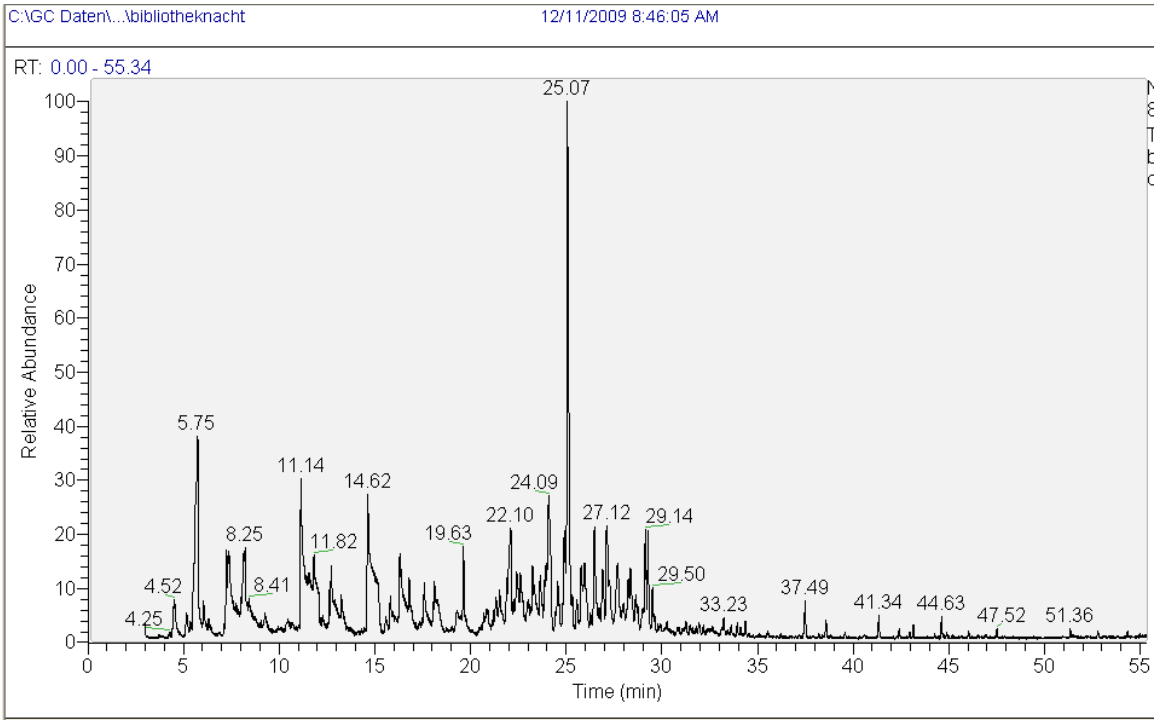

Abbildung 8 Chromatogramm: Buch aus Bibliothek, Über-Nacht-Messung

Den Peak mit höchster Intensität stellt **α–Limonen** (29) dar, mit einer Retentionszeit von 25.07 Minuten. Limonen ist eine farblose Flüssigkeit mit einem Orangen-Zitrus-Geruch, eine Substanz die in vielen Lacken und in Reinigungsmittel erhalten ist. Der zweite höchste Peak, stellt **Essigsäure** dar, ein bekanntes, flüchtigers Papierabbauprodukt und ubiquitärer Bestandteil der Troposphäre. Bei Rt 8.41 findet mann **4-Methylcyclohexanol,** das auch als Reinigungsmittel verwendet werden kann und bei Rt 11.14. einem größeren Peaks, **Toluol**, das im Printing Shops nachgewiesen wurde. Der Peak bei Rt 14.62 stellt **Furfural** dar, ein bekanntes flüchtiges Papierabbauprodukt. **α–Pinen**, Rt 19.63 wird aus Holz und Holzwerkstoffen (insbesondere von Nadelholz), die in Innenräumen verbaut sind emittiert. **Nonanal** mit Rt 29.14, ist enthalten in: Duft-, Riech-, Aromastoffen, Parfüms und Kolophonium (Rosin). Bei Rt 44.63 erscheint ein kleiner Peak von (+)-Longifolen, das als Lösemittel in Lacken, Farben und z.T. in Klebstoffen eingesetzt wird und auch in Dispersionsklebstoffen vorkommt.

4.2. Untersuchungen der Innenraumluft des Antiquariates "Schaden"mittels SPME-GC-MS

Zur Probensammlung wurde das Antiquariat ``Schaden``, ein kleines Geschäft im Zentrum von Wien, ausgesucht.

Die SPME-Fasern wurden nacheinander in das GC eingespritzt und mittels GC-MS Technik konnten die einzelnen Riechstoffe getrennt und analysiert werden.

Die gewonnene Chromatogramme wurden mit den Daten der früheren Analyse des isolierten Buches, sowie mit mehreren Studien wie: „On the Odour of Old Books“ [16] und „On the Smell of Old Books“ [24] verglichen.

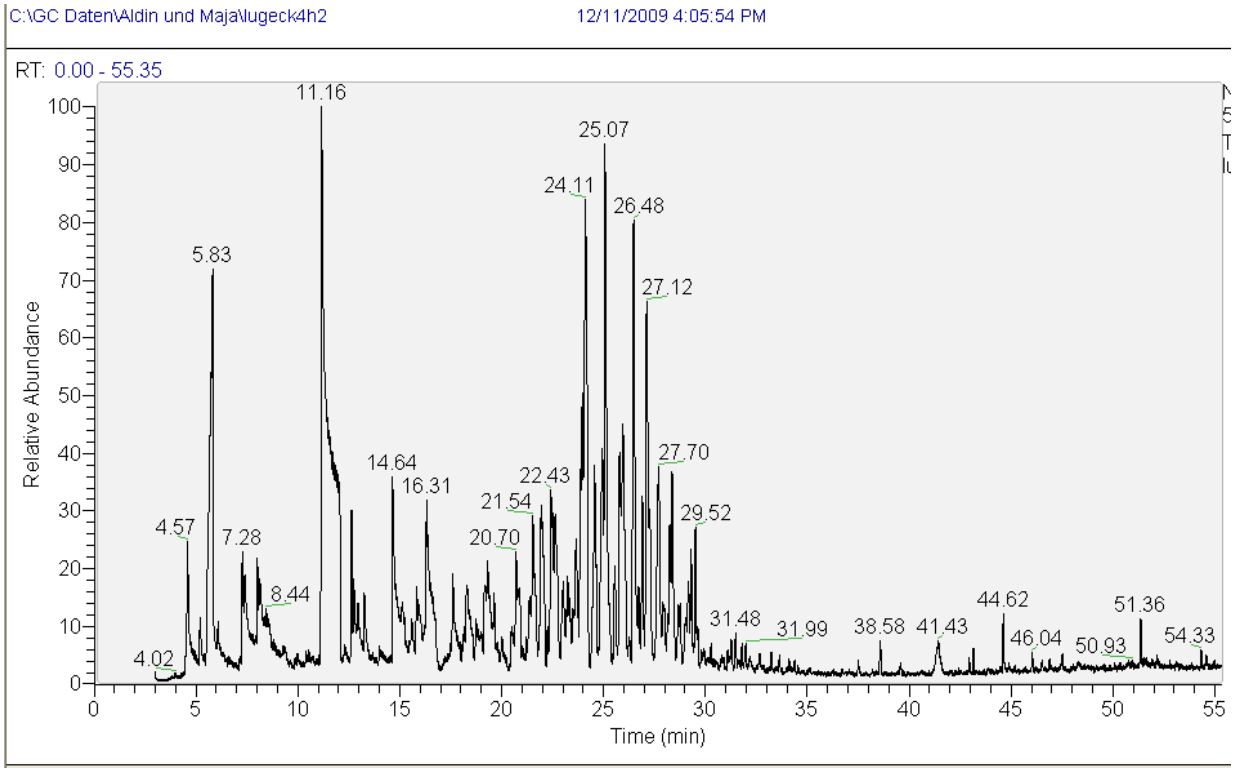

Abbildung 9 Chromatogramm der 4h Messung

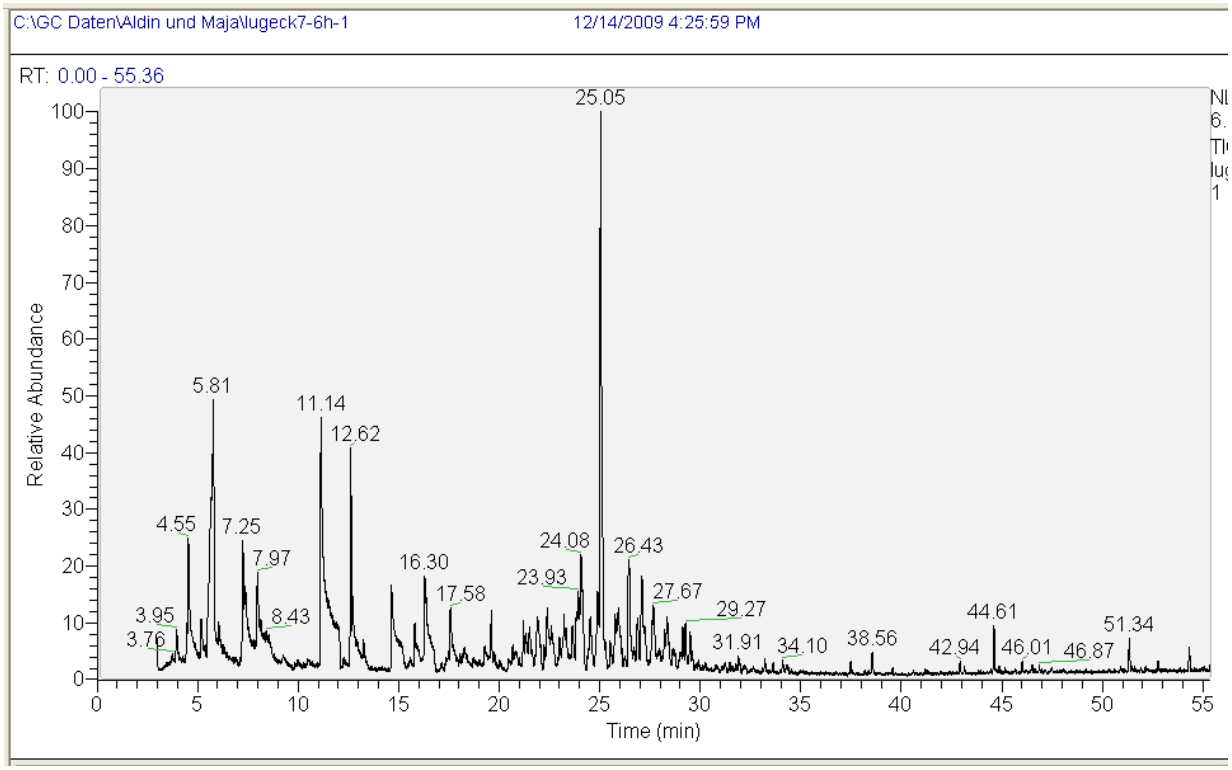

Abbildung 10 Chromatogramm der 6h Messung

In der folgenden Tabelle sind die wichtigsten Aromastoffe der den bei Messungen im Antiquariat (vier und sechs Stunden) zusammengefasst, die bei der HS-SPME-GC-MS gesammelt und aufgezeichnet wurden.

	Rt	Substanz	Synonym	Geruch
1	4,5	2,4,4-Trimethyl-2-pentanamin	Tert-octylamin	
2	4,57	1,3-Pentadien	Piperylen	
3	5,13	2-Butyltetrahydrofuran		
4	5,5	1-Pentanol	Amylalkohol	
5	5,57	Ethanol		
6	5,62	2-Methylfuran	Sylvan	
7	5,69-5,82	Ethansäure	Essigsäure	stechend riechend
8	5,77	Pentandial	Glutaraldehyd	scharf, unangenehm
9	6,05-6,08	Heptansäure	Önanthsäure	ranzig, unangenehm
10	7,24-7,28	Benzen	Benzol	benzolartig
11	8,15	Propansäure	Propionsäure	stechend, unangenehm
12	8,41	4-Methylcyclohexanol		
13	8,42-8,45	Pentanal	Valeraldehyd	süßlich, ranzig
14	11,13-11,16	Toluen	Toluol	süßlich-stechend
15	12,72	Hexanal	Capronaldehyd	ranzig
16	13,25	Essigsäurebutylester	Butylacetat	angenehm, fruchtartig
17	14,61-14,64	2-Furfural	α- Furol	mandelartig
18	15,58	Furfuranol	2-Furanmethanol	
19	15,81-15,83	Ethylbenzol	α -Methyltoluol	benzolänhlich
20	16,31-16,39	Xylen	Dimethylbenzol	aromatisch
21	16,94	Butansäure	Buttersäure	unangenehmes Geruch nach Erbrochenem
22	17,24	Methoxy, Phenyloxim		
23	19,58-19,63	α -Pinen		kieferähnlich
24	21,52	Undecan		
25	21,83	α -Terpinen		holzig,zitronenartig
26	21,88-21,95	Benzaldehyd		bittermandelartig
27	22,25	Myrcen		
28	22,42	Phenol		durchdringend
29	25,07	α -Limonen		zitronenartig
30	25,32	1,8-Cineol	Eucalyptol	
31	29,13-29,15	Nonanal	Pelargonaldehyd	fettig, rosig
32	31,09	Benzoesäure		
33	31,9	1,7,7-Trimethyl-bicyclo[2.2.1]-heptane-2-on	Kampfer	kampferartig, würzig
34	33,2	(+)-Neomenthol		pfefferminzartig
35	38,58	2-Myrcenol		zitrusartig
36	39,57	Methylnaphthalin	Naphthalin	Geruch nach Teer
37	44,62	(+)-Longifolen		

1. 2,4,4-Trimethyl-2-pentanamin Dabei handelt es sich um ein niederes Amin, eine katabolische Komponente von Protein und Kollagen des tierischen Bindegewebes, welches als Binde Material Verwendung findet [16].

2. 1,3-Pentadien wird bei der Herstellung von C_5-Kohlenwasserstoff oder Erdöl-Harzen verwendet und weist auf eine geringe Belastung der Umwelt und am Arbeitsplatz hin [17].

3. 2-Butyltetrahydrofuran wird verwendet als ein bedeutendes Industrie-Lösungsmittel und ist für seine einzigartige Kombination von nützlichen Eigenschaften bekannt. THF ist ein farbloser flüchtiger cycloaliphatischer (5-gliedrige) Ether mit charakteristischem Geruch [18].

4. 1-Pentanol wird zur Herstellung von Pharmazeutika, Kosmetika, Farb- und Gesmackstoffen verwendet und ist zudem in Desinfektionsmittel erhalten. Bei dieser Substanz handelt es sich um eine farblose, unangenehm riechende Flüssigkeit, welche große Bedeutung bei der Herstellung von Fruchtethern hat und in der Parfümerie als Lösungsmittel für Fette und Öle Einsatz findet [19]. Des Weiteren wurde es in Körperausdünstungen und in Innenraumluftmessungen gefunden [20].

5. Ethanol ist eine leichte entzündliche, klare, farblose Flüssigkeit mit würzigem Geruch, umfangreiche Verwendung in Pharmazie und Industrie, zur Herstellung alkoholischer Getränke, gutes Lösungsmittel für Harze, ätherische Öle und Wachse. Des Weiteren wird der Alkohol auch als Desinfiziens verwendet [20, 21].

6. 2-Methylfuran ist eine brennbare, leicht flüchtige Flüssigkeit mit Ether-ähnlichem Geruch. Methylfuran ist eine Substanz, die in Spuren im Gasspektrum von Biogasen und in MVOC Innenraumluftmessungen nachgewiesen wurde [22, 23].

7. Essigsäure ist ein bekanntes flüchtiger Papierabbauprodukt (aus Lignin) das typisch für altes Papier und für Gelbfärbung des Papiers ist [24, 16].

Als Stoffwechselprodukt aller Lebewesen kommt es in der Umwelt vor. Essigsäure ist ein ubiquitärer Bestandteil der Troposphäre und signifikant für die natürliche Azidität des Regens und des Wolken- und Nebelwassers insbesondere in kontinentalen Regionen.

Essigsäure dient zur Synthese von Arznei-, Pflanzenschutz- und Konservierungsmitteln, Farb- und Riechstoffen und gilt zudem als Hilfsmittel in der Färberei [21].

8. Pentandial-(Glutaraldehyd) dient zur Desinfektion und kann im entsprechenden medizinischen Umfeld eine Rolle als Luftschadstoff spielen. Wird auch als Zusatz in Reinigungsmittel verwendet [20, 21].

9. Heptansäure wird verwendet als Aromastoff, Stabilisator in Schmiermittel und findet Einsatz als Hydraulikflüssigkeit.

BTEX-Aromaten (10. Benzen, 11. Toluol, 12. Ethylbenzol, 13. Xylen) Der größte Teil der produzierten BTEX wird in Benzinen zur Erhöhung der Oktan-Zahl eingesetzt. BTEX-Aromaten werden als Lösungsmittel für Kautschuk, Fette, Harze und Öle verwendet [21].

11. Toluol BTEX-Aromaten-Toluol wurde in Printing shops nachgewiesen [26].

Eines der VOCs (flüchtige organische Verbindungen), die normalerweise in Innenräumen vorkommen [27].

12. Ethylbenzol, BTEX-Aromaten

13. Xylen BTEX-Aromaten

14. Propionsäure wird verwendet als Konservierungsmittel in der Lebensmittelindustrie und zur Herstellung von Herbiziden.

15. 4-Methylcyclohexanol findet Verwendung als Lösungsmittel für Cellulose-Ester und Ether, als Antioxidans in Mischungen von Seifen und Reinigungsmitteln und auch als Schmiermittel in den Kunstseidenindustrie [25].

16. Pentanal, das Abbauprodukt des Lignins, das typisch für altes Papier und für Gelbfärbung des Papiers ist [16, 24]. Pentanal wird in verschiedenen Aromen (z. B. Fruchtaromen), sowie als Vulkanisationsbeschleuniger verwendet. Vulkanisation ist ein chemisch-technisches Verfahren, bei dem Kautschuk unter Einfluss von Zeit, Temperatur und Druck gegen atmosphärische und chemische Einflüsse, sowie gegen mechanische Beanspruchung widerstandsfähig gemacht wird. Synthesekautschuk wird für Autoreifen, Gummiformteile und Klebstoffe verwendet.

17. Hexanal wird in der Natur gebildet, wenn Fettsäuren (18 C) aus der Zellwand durch Lyasen in Hexan zerlegt wird. Durch eine Oxidation entsteht danach das Hexanal. Ist ein Abbauprodukt des Lignins, typisch für altes Papier und für die Gelbfärbung des Papiers verantwortlich [16]. Hexanal ist in vielen Anstrichmitteln, in Linoleum und in Arzneimitteln enthalten. Linoleum wird für Tapeten, vereinzelt als Belag für Möbelstücke (Tische, Schränke, Pinnwände) verwendet. Des Weiteren findet Hexanal auch in der Riechstoffchemie Verwendung [28].

18. Butylacetat Dabei handelt es sich um eine der VOC-Substanzen, welche normalerweise in Innenräumen vorkommt [26]. Es ist in Druckfarben enthalten [29].

19. Furfural ($C_5H_4O_2$) ist eine klare, gelbliche Flüssigkeit mit typisch mandelartigem Geruch. Im Zuge der Produktion von Viskosefasern, wird bei der Holzkochung von Buchenholz Furfural durch Zweifachdestillation freigesetzt. Das garantiert die Beseitigung sämtlicher Verunreinigungen und gewährleistet ein Produkt höchster Reinheit [30]. Furfural ist ein bekanntes flüchtiges Papierabbauprodukt, dessen Emission direkt mit der Papierazidität korrelieren kann [24]. Eine Nutzung findet in Form von Holz als Baustoff und Brennstoff statt. Der Celluloseanteil wird zur Papierherstellung verwendet. Lignin ist dabei ein Abfall- und Störstoff, der in der verwendeten Lignocellulose in möglichst geringer Menge vorliegen sollte. In verschiedenen Pilotprojekten wird versucht, Lignocellulose aus Getreide, Stroh, Holz, Papier und cellulosehaltigen Abfällen, als nachwachsenden Rohstoff für unterschiedliche chemische Grundstoffe zu verwenden. Furfural ist eine der Substanzen die im alkoholischen Extrakt von Kiefernholz (*Pinus sylvestris L.*) identifiziert wurden [31].

20. Furfuranol Es ist auch unter den Synonymn Furfurylalkohol, 2-Furylmethanol oder 2-Furancarbinol bekannt. Furfuranol ist eine heterocyclische organische Verbindung mit einer Hydroxymethyl-Gruppe. Es ist eine transparente, farblose bis hellgelbe Flüssigkeit mit Bittermandel Geruch und wird braun, hellgelb oder rot, wenn es länger der Luft ausgesetzt ist. Furfuranol findet Verwendung als Lösungsmittel, aber wird in erster Linie als Zutat bei der Herstellung von verschiedenen chemischen Produkten wie Gießereiharzen-, Klebstoff-und Netzmittel verwendet. Es kann auch benutzt werden, um alle Arten von Furanharz zu produzieren. Furfuranol kann in der Gummi-, Pestizid-und Gießerei-Industrie verwendet werden [32].

21. Buttersäure riecht äußerst penetrant und widerlich nach "Erbrochenem". Sie wird verwendet zur Herstellung von Buttersäureestern, Cellulosebutyrat (witterungsbeständiger und schlagfester Kunststoff), Medikamenten und Schädlingsbekämpfungsmitteln [20].

22. Methoxy-phenyloxim wurde auch in der Studie [24] über Geruch von alten Büchern gefunden.

23. α–Pinen ist ein bicyclisches Monoterpen, welches den Hauptbestandteil der Terpentinöle, die aus dem Harz von Pinusarten durch Wasserdampfdestillation gewonnen werden, darstellt. Pinen wird nicht nur von den lebenden Pflanzen emittiert, sondern auch aus Holz und Holzwerkstoffen (insbesondere von Nadelholz), die in Innenräumen verbaut sind [20]. Außerdem ist es auch im Kolophonium (Rosin) enthalten. Resine (Extraktstoffe aus dehydrierten Naturharzen) werden als Zwischenprodukt in der chemischen Industrie, z. B. als Synthesekautschuk (für Autoreifen, Gummiformteile, Klebstoffe), Schiffsfarben oder zur Pigmentherstellung für Druckfarben verwendet [33].

24. Undecan, enthalten in Rosin [24]. Undecan ist ein Bestandteil des Erdöls [19]. Erdöl ist ein natürlich vorkommendes Gemisch von Kohlenwasserstoffen sehr unterschiedlicher Zusammensetzung [21].

25. α–Terpinen findet hauptsächlich Verwendung als Bestandteil ätherischer Öle und Aromen. Es gilt als Reinstoff und ist Zwischenprodukt in der organischen Synthese von Terpenen und Mischpolymerisaten, die zu Harzen und Lacken weiterverarbeitet werden. Es ist auch Bestandteil biologisch abbaubarer Farbentferner sowie abbaubarer antimikrobieller Reinigungsmittel [34].

26. Benzaldehyd ist ein Abbauprodukt des Lignins, welches typisch für altes Papier und auch für dessen Gelbfärbung des Papiers ist [24, 16]. Benzaldehyd riecht intensiv nach bitteren Mandeln - angenehm, aromatisch, kräftig und süß und wird als Lösungsmittel zur Herstellung von Farbstoffen verwendet [35].

27. Myrcen ist ein acyclisches Terpen, das außer von Nadelhölzern auch von Luzernen freigesetzt wird und in der Atmosphäre vorkommt [20].

28. Phenol ist wesentlich für die Herstellung von Farbstoffen, Holzschutzmittel, Phenolharzen und Klebstoffen [21]. Gelegentlich findet man es auch in Desinfektionsmitteln [20].

29. α–Limonen ist eine farblose Flüssigkeit mit einem Orangen-Geruch, enthalten in ätherischen Ölen. Limonen gehört zu der Gruppe der Terpene und ist Bestandteil vieler Farben und Lacke. Dieses Terpen gehört nach den Ergebnissen des Umwelt-Survey des Bundesgesundheitsamts zu den in Innenräumen mengenmäßig bedeutsamsten Verbindungen [20]. Heute wird es vorwiegend als biogenes Lösungsmittel verwendet und dient als Reiniger und Verdünnungsmittel, beispielsweise in der Lackindustrie. Häufiger VOC in Printen Shops [26].

30. 1,8-Cineol (Eucalyptol) kampferartiger Geruch [36]. Es findet wegen seines würzigen und kampferartigen Geruchs Einsatz in der Lebensmittelindustrie [37].
1,8-Cineol wird einerseits bei Atemwegserkrankungen des Menschen verwendet, andererseits kommt es als Aromastoff in der Parfümindustrie zum Einsatz.

31. Nonanal ist enthalten in: Duft-, Riech-, Aromastoffen und Parfüms [28]. Sein Geruch wird beschrieben als: prägnant, komplex, oszillierend; blumig-rosenhaft, citrusartig, Iris- und Orangenbütennote, fettig (jedoch ohne Schweißnote), wachsig.
Dieser Aldehyd passt in blumige Kompositionen und Phantasiebouquets, insbesondere in solche mit Rosen-, Cassien- und Liliencharakter. Auch in Orangenblüten- und Citruspräparationen gilt es passend. Es ist nicht so dominant im Geruchseindruck wie die anderen Fettaldehyde, weshalb mit Nonanal auch höhere Einsatzmengen erzielt werden [35]. Nonanal ist in Kolophonium (Rosin) enthalten [24, 38]. Kolophonium ist ein gelbes bis braunschwarzes natürliches Baumharz und wird als Bestandteil von Druckfarben, Lacken, Klebstoffen, Seifen, Papier-„Sizing“, Flussmittel und Siegellack verwendet.

32. Benzoesäure ist eine der gängigsten Konservierungsstoffe der Lebensmittelindustrie. Als Ester und in freiem Zustand ist Benzoesäure in vielen Harzen (besonders Benzoeharz) und Balsamen (Tolubalsam, Perubalsam) verbreitet. Es dient als Zwischenprodukt für die Farbstoff- und Parfümherstellung und als Konservierungsmittel [39].

33. 1,7,7-Trimethyl-bicyclo[2.2.1]-heptan-2-on (Kampfer).

Der Großteil von Naturcampher stammt aus dem ätherischen Öl von Holz, Wurzeln, Zweigen und Blättern des tropischen Kampferbaumes *Cinnamomum camphora* (L. SIEB). Kampfer zählt allgemein zu den Luftschadstoffen in Innenräumen [20]. Sein Geruch wird beschrieben als: durchdringend, intensiv, aromatisch, medizinisch, holzig. Kampfer wird aufgrund des angenehmen Geruchseindrucks im kosmetischen Bereich verwendet [35]. Hauptsächlich großtechnische Anwendungen findet Kampher in der Celluloidproduktion sowie als Weichmacher für Celluloseester. Kampfer ist im Kolophonium (Rosin) enthalten, welcher als Klebstoff oder für Pigmentherstellung für Druckfarben verwendet wird [16].

34. (+)-Neomenthol Sein Geruch wird als angenehm, erfrischend, süß, "minzig", pfefferminzartig, etwas stechend, mit ausgeprägtem Kühleffekt bezeichnet. Wurde in verschiedenen ätherischen Ölen gefunden [35].

35. 2-Myrcenol- wird als Duftstoff verwendet [40].

36. Methylnaphtalin- ist ein kristalliner weiβer Feststoff mit charakteristischem Geruch und wir hauptsächlich aus dem Steinkholenteer gewonnen. Naphthalin ist eine wichtige Grundchemikalie für die Herstellung von Farbstoffen, Insektiziden und Arzneimitteln. Außerdem dient Naphthalin der Herstellung von Tetralin und Decalin, welche Lösungsmittel für Lacke darstellen [41].

37. (+)-Longifolen wird aus Koniferen oder bei der Zellstoffproduktion (Sulfatterpentinöl) gewonnenen. Es zeigt eine farblose bis schwach gelbliche Färbung mit angenehmem Geruch. Terpentinöl wird als Lösemittel in Lacken, Farben und z.T. in Klebstoffen eingesetzt und kommt auch in Dispersionsklebstoffen vor. Die Hauptinhaltsstoffe sind Pinen und Caren und die werden darüber hinaus als Ausgangsprodukte für andere Terpenverbindungen eingesetzt. Das so genannte "Gereinigte Kienöl" besitzt ähnliche Inhaltsstoffe und ein gleiches Anwendungsspektrum [42]. Wird auch als Lösungs- und Reinigungsmittel verwendet. [36].

5. SCHLUSSBETRACHTUNG

5.1. Allgemeines zu den Düften des Antiquariates

Das Ziel dieser Arbeit war es, den Duft in den Räumen des Antiquariates zu erfassen und möglichst viele Aromastoffe nachzuweisen.

Alte Bücher zeigen einen charakteristischen Geruch, welcher im Antiquariat Schaden untersucht wurde. Für diesen typisch staubigen, muffigen, schimmeligen und Papier-ähnlichen Geruch sind auch die Vielzahl von Materialien, die für Buch Produktion verwendet werden (Papier, Tinte, Klebstoff...), mitverantwortlich. Zu dem Geruch tragen auch andere Substanzen, die von Möbeln (Holz, Lack...), Reinigungsmitteln, Parfümkomponenten und andere, welche typisch für Innenräume sind, bei.

Für diese Untersuchungen wurde die HS-SPME-Methode in Kopplung mit GC-MS verwendet, da sich diese Methode in Aromastoffanalyse als leistungsfähig gezeigt hat. Die Ergebnisse wurden mit Hilfe von Datenbanken erarbeitet.

Zur weiteren Interpretation waren bestehende Arbeiten zum Thema ältere Bücher (Literaturangaben [16, 24, 26]) sowie Publikationen auf dem Gebiet der Aroma- Duft- und Riechstoffchemie von großem Nutzen.

Die im Chromatogramm aufgetrennten Peaks wurden einzeln ausgewertet und mit bekannten Daten verglichen.

5.2. Interpretation der Analysendaten mit charakteristischen Substanzen

Von den mehr als 200 nachgewiesenen Substanzen wurden jeveil 40 ausgesucht, die die größte prozentmäβige Übereinstimmung mit den Datenbanken zeigten und die mit älteren Arbeiten verglichen werden konnten.

In den Chromatogrammen wurden flüchtige Abbauprodukte mit wichtigen Eigenschaften für die Erhaltung von historischem Papier: Harz, Lignin und Carbonylgruppen, mittlere und höhere Aldehyde und Alkylcarbonsäuren gefunden.

Da man die Messungen im Räumen, wo sich täglich Leute aufhalten, durchführte, wurden auch Substanzen ermittelt, die von Reinigungsmitteln, Parfüms und/ oder Speisen stammen.

Es ist signifikant, dass sich die Chromatogramme nur in den Konzentrationen der einzelnen Komponenten, nicht aber drastisch in ihrer Gesamtzusammensetzung, unterscheiden. Die charakteristischen Substanzen finden sich sowohl im vier- als auch in dem sechsstündigen Chromatogrammen vor.

Im Chromatogramm des isolierten Buches zeigt Limonen den höchsten Peak. Die anderen Substanzen sind auch bei Chromatogrammen aus dem Antiquariat nachzufinden.

Die meisten Substanzen, die detektiert wurden, sind für die Alterung der Bücher charakteristisch. Des Weiteren wurden auch Verbindungen gefunden, die in Materialien für die Buchproduktion (Tinte, Klebstoff...) verwendet werden.

Pentanal, Hexanal, Benzaldehyd, Essigsäure sind Abbauprodukte des Lignins, das typisch für altes Papier und für Gelbfärbung des Papiers ist [16, 24]. Furfural ist ein bekanntes flüchtiges Papierabbauprodukt, dessen Emission direkt mit der Papierazidität korrelieren kann [24]. Substanzen, wie Nonanal, α-Pinen, Kampfer, Undecan, sind in Kolophonium enthalten [24, 38]. Kolophonium ist ein gelbes bis braunschwarzes natürliches Baumharz und wird als Bestandteil von Druckfarben, Lacken, Klebstoffen, Seifen, Papier-Sizing, Flussmittel und Siegellack verwendet. Außer Limonen sind noch weitere Reinigungs- und Desinfektionsmittel wie Ethanol, Glutaraldehyd und 4-Methylcyclohexanol, vertreten.

Andere Substanzen wie zB. Butylacetat kommen normalerweise in Innenräumen vor [26].

6. ZUSAMMENFASSUNG

Da Wiener Antiquariate und die Tradition des Buchhandels in Österreich schon seit dem siebzehnten Jahrhundert bekannt sind, wurde in dieser Arbeit im Rahmen eines Projekts des Wiener Wissenschafts-, Forschungs- und Technologiefonds (WWTF) – "*Haptic and Olfactory Design, Resources for Vienna's Creative Industrie",* der Geruch, d.h. die Duftstoffe, die in einem Antiquariat vorkommen, untersucht. Das Ziel dieser Arbeit war es, den Duft des Antiquariates zu erfassen und möglichst viele Aromastoffe nachzuweisen, um zu erfahren wie eine solche Räumlichkeit riecht.

Im Antiquariat "Schaden" wurden die Proben mittels SPME-Methode eingesammelt und dann mit Hilfe der GC-MS Technik getrennt und analysiert. Die im Chromatogramm getrennten Peaks wurden einzeln ausgewertet und mit Hilfe von bekannten Datenbanken identifieziert.

Zur weiteren Interpretation waren bestehende Arbeiten zum Thema ältere Bücher (Literaturangaben [16, 24, 26]) sowie Publikationen auf dem Gebiet der Aroma-, Duft- und Riechstoffchemie von großem Nutzen. Die Chromatogramme der vier- wie auch der sechsstündingen Messungen unterscheiden sich nur in den Konzentrationen der einzelnen Komponenten, nicht aber drastisch in ihrer Gesamtzusammensetzung.

In den Chromatogrammen wurden flüchtige Abbauprodukte mit wichtigen Eigenschaften für die Erhaltung von historischem Papier, von Harzen und Lignin wie Carbonylverbindungen, mittlere und höhere Aldehyde und Alkylcarbonsäuren gefunden.

Für den typisch staubigen, muffigen, schimmeligen und papier-ähnlichen Geruch sind auch die Vielzahl von Materialien, die für Buch-Produktion verwendet werden (Papier, Tinte, Klebstoff...), mitverantwortlich.

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