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Flavor of Aromatic Fruits and Spices from the Tropical Rainforest – A Field Study

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Abstract: Consumers demand new and improved flavor sensations, which cannot always be fulfilled by traditional flavors from known fruits. Inspiration for new developments in flavors may be generated from plant material originating from the tropical rainforests. The tropics house 80 to 90% of all plant species and the aromatic aspects of most of these are entirely unknown. Field methods for the collection of samples, the isolation and conservation of the aroma compounds in very remote areas are presented. The overall flavor profile and the key aroma compounds of selected fruits and 'garlic' barks collected from the Gabonese tropical rainforest 'forêt des Abeilles' are described.

Keywords: Field methods · Flavor chemistry · Headspace analysis · Tropical fruits

Background

As information technologies and ease of travel continue to advance rapidly, people from around the world are enjoying the ability to share other cultures. More than ever, people realize that in other parts of the world there are foods and flavors that they have never experienced before. This awareness is influencing cultural eating habits the world over. Whereas, in the past, people generally were not very adventurous in their eating habits, today openness toward new foods, tastes and trends prevails. The interest in unique and exotic food fuels itself, consumers today are demanding 'new' and 'improved' flavor sensations at a much greater rate. This new openness to global tastes and cultural sharing challenges the innovative abilities of the flavor industry. Scientists have always considered nature as a valid source of inspiration. The leaf of a lotus plant taught the engineer about self-cleaning surfaces, mushrooms taught the pharmacologist about antibacterial substances, a blade of grass taught the architect about a stable and flexible construction with minimal weight. Nature is our first source of innovation. Commercially available fruits *e.g.* apples or strawberries, are the result of a breeding process over decades. Increase in size and storage stability has often been paid for with a reduction in aroma. In our quest for new inspiration we left the 'industrialized' and at the same time uninteresting and well investigated products aside. They are rarely a valid target for new developments. We have to leave our laboratory to experience truly new aromas and tastes.

The Tropical Rainforest – An Unexplored Taste Treasure?

In the constant hot humid climate of the tropics with average annual temperatures of 26 to 27 °C and at least 2000 mm of rainfall evenly spread all over the year, an extraordinary biological structure has evolved. What we today describe as the tropical rain forest has developed over millions of years under quite constant climatic conditions. It is one of the most complex, but very fragile ecosystems. The favorable humid and warm climate is in strange contrast to the poor soil. Competition for light and nutrients is fierce. This has led to an incredibly huge biodiversity. The tropical rainforests cover only *ca.* 11–13% of all landmass but houses approx. 80–90% of all animal and plant species. The majority of all these species, – *ca.* 70 to 80%, – occupy the canopy region of the rain forest – one of the least explored and most inaccessible places of the world. During a four week period we had the opportunity to investigate unexplored flavors at the mission 'Makandé 99' to the tropical rainforest around 'Camp Makandé' in the region 'forêt des Abeilles' in Gabon. An overview of the mission, which was organized by Pro Natura International, is given in [1]. We hoped that the high biodiversity in this region would also be reflected in the richness of aromatic plants and fruits.

Collection, Evaluation and Identification of Samples

In the past, several systems have been developed to access the canopy region. A very advanced tool to access the canopy was developed in the group of Prof. Hallé at the University of Montpellier. It basically consists of a maneuverable hot air balloon (Fig. 1). With its air volume of 8500 m³ it can carry an inflatable trian-

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gle, which can be positioned anywhere on the tree tops, and allows the scientists to investigate the canopy. This system keeps interference with the ecosystem small, and provides exceptional mobility and access. Also samples from the middle layer and the under-story were collected. Co-operation with ethnobotanists gave valuable insight into the eating habits, and use of fruits and plants by the local people. In the field, our team recorded a flavor profile of each fruit or botanical using the consensus technique [2]. The edible parts of the plants were chewed to obtain an aroma and taste impression, but they were not swallowed.

Fig. 1. Early in the morning, the maneuverable hot air balloon is prepared for take off

Field Methods

Fruits or other plant parts are in constant development and once fully ripe deteriorate quickly. Preserving samples using *e.g.* sterilization interferes with the aroma compounds. Heat-induced reactions could take place, and may destroy the authentic, delicate aroma. Everybody can easily experience this by simply comparing the aroma from a fresh pineapple to a canned one. Also a drying process can cause dramatic changes as described *e.g.* for basil [3] and parsley [4]. Potential aroma compounds may be very volatile, with boiling points near room temperature like acetaldehyde and dimethyl sulfide. Others react with the sample matrix by enzymatic or non-enzymatic reactions. Often one method can only cover some aspects of the whole complex mixture of an aroma, which makes it advisable to apply complementary sampling techniques. Taking into consideration the limitations of remote places, sampling methods must be mobile, easy to handle and robust. Therefore, many of the sophisticated techniques for the isolation of aroma compounds, see the review of Teranishi and Kint [5], which require huge equipment (vacuum distillation, cryotrapping), cannot be applied. Furthermore, the field researcher has the problem that the amount of sample is limited. Sometimes just one or two fruits of one species are available, which requires treatment with utmost care. Consequently we developed appropriate field methods for sampling and conservation of the aroma molecules.

Headspace Methods

The air surrounding an aromatic sample is called the 'headspace'. It contains the aroma compounds of the sample. Headspace techniques collect and analyze the aroma compounds in the air. This can be done directly (statically) or after enrichment on an adsorbent (dynamically). Field headspace techniques require the trapping of volatile compounds on an adsorbent. Charcoal or porous polymers like Porapak, Tenax or others are used as adsorbents. A general review on the various techniques in use is given by Bicchi and Joulain [6] and Knutsen *et al.* [7]. Air and water pass through the adsorbent, while the aroma compounds are trapped and remain on the filter. They can be extracted from the filter by thermodesorption or by elution with organic solvents. Although the concentration of aroma compounds in the headspace is low, the tremendous progress in instrumental analysis during the past 30 years allows the identification and structure elucidation of compounds in the nanogram to microgram range. This enables headspace techniques to be used in the identification of trace compounds. For example, using thermodesorption-GC/MS to elute the volatiles from a filter requires only *ca.* 1 ng of a compound to get a mass spectrum for interpretation. With increasing sensitivity in the future even less material will be needed.

Headspace Trapping and Extraction – An Example

We used an adaptation of the method developed by R. Kaiser and coworkers [8] for the isolation of volatile compounds from flowers. The headspace trapping and extraction of the aroma of *Delpydora macrophylla* Pierre fruits is described in detail below to give an impression (see Fig. 2): The fruits were cut into two pieces, and laid on a sieve, which was placed in a glass container. The air from the container was pumped at 40 ml/min (3 h) through a headspace filter containing 5 mg of Porapak Q. Finally, the volatiles trapped on this filter were eluted using 30 ml acetone/hexane 1:10. The headspace extract was sealed and transported to our laboratories for analysis by GC/MS and GC-olfactometry. The air, which was flushed through the headspace of the sample, was filtered through a Porapak Q trap before entering the sample container to avoid contamination from the environment.

Headspace Trapping and Thermal Desorption

To get more concentrated samples, a headspace adsorption-thermodesorption method was also applied [9]. A tube (170 mm x 4 mm) filled with *ca.* 100 mg of Tenax® as adsorbent was used. After collecting the aroma compounds as described above, the tube was sealed and put into storage. Back in the laboratory, the volatiles were thermally desorbed, concentrated by a cryofocusing system and then injected into a GC. Employing this method, the dilution of the sample with solvent is avoided, which yields a 10 to 30 fold increase in concentration, allowing the identification of trace components.

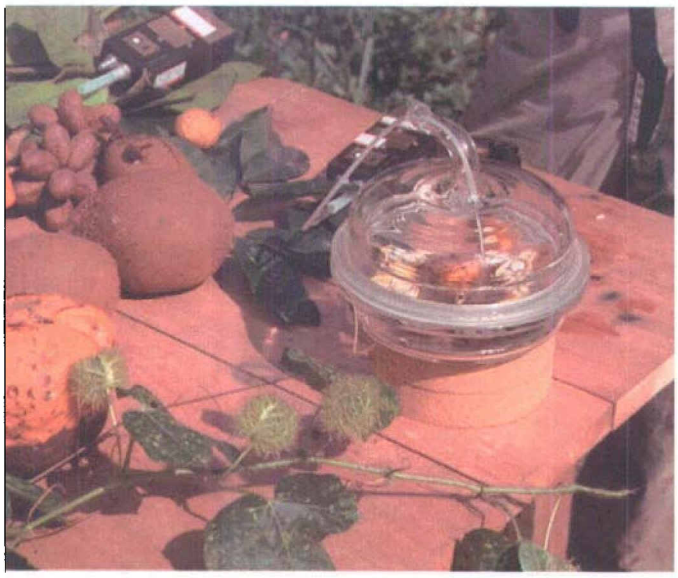

Fig. 2. The aroma of the fruits of *Delpydora macrophylla* Pierre is trapped on Porapak Q.

Solvent Extraction

Only a small part of the flavor compounds are released during headspace trapping. Most of the volatiles are still present in the fruit pulp. Where enough material was available, we prepared extracts from the pulp. Typically the fruits were peeled and the seeds removed. The flesh was added to a saturated solution of calcium chloride to inhibit enzymatic activity, and then chopped. The fruit mix was extracted twice with the same amount of a mixture of acetone and hexane 1:10. The resulting layers were separated in an extraction funnel and the organic solution was filled into aluminum bottles. The bottles were stored in a refrigerator before transportation to the laboratory. The aroma compounds were stable in the extract, no fermentation was observed. The volatiles were isolated in the laboratory by high-vacuum transfer [10]. GC/MS and GC-olfactometry were performed on the extract as well as on fractions obtained by chromatography on silica gel, employing the method of Widder *et al.* [11].

Aromas of Selected Samples

In one of our first walks through the under-story of the rainforest, we found a small three-meter high shrub, with hairy orange yellow fruits (Fig. 3) emerging directly from the stem. The species was identified as *Delpydora macrophylla* Pierre, a member of the Sapotaceae family. The appearance of the fruit was reminiscent to that of a gooseberry, but much bigger. However, it was quite different from gooseberry when opened, possessing five hard seeds surrounded by a soft white fruit pulp. The hairy skin of this fruit was very leathery. A very delicate peachy, lactonic smell with sulfurous and meaty aspects was emitted from *D. macrophylla* Pierre. On tasting we experienced a sweet, fruity and succulent impression. Since only a few fruits were found, we applied the above mentioned headspace-extraction technique. A fruity aspect from *D. macrophylla* Pierre was identified as being contributed by linalool (floral, fruity), methyl salicylate (medicinal, red fruit) and the aldehydes nonanal and decanal (orange). Its primary aroma was modified by terpenic, campherous notes exhibited by α-pinene and camphor. Finally, freshness was added by *trans* and *cis*-2-hexenal, hexanal and hexanol.

Abundant in this area of rainforest were the grape-sized orange fruits from *Dacryodes klaineana* H. J. Lam, a member of the Burseraceae family. *D. klaineana* H. J. Lam is a medium high tree, which is part of the middle layer (10–20 m) of the tropical rain forest. It is mainly found in areas of continuous canopy cover, where the herbaceous growth in the under-story is sparse. What we called the 'mini-mango' was a small yellow brownish fruit with leather-like skin (Fig. 4). *D. klaineana* H. J. Lam exhibits a large seed surrounded by a very thin layer of edible orange flesh. The aroma is very delicious sweet-sour, reminiscent of mango but more transparent. Also woody, terpenic and green notes are present. In the camp we separated the flesh from the seeds and peel, and extracted the volatiles as previously described. We were quite surprised that the overall fruity aroma of *D. klaineana* H. J. Lam is almost entirely caused by terpenes, which is unusual for most of the fruits that we have seen before (Fig. 5). α-Pinene (**1**, 6.9 area%) and β-pinene (**2**, 8.1%) with pine-like and woody-mango aspects are dominant. The hesperidic freshness is due to the quite high amount (5%) of *cis*-β-ocimene (**3a**) and *trans*-β-ocimene (**3b**), which is modified by oxygenated monoterpenes with woody and fruity notes like terpinene-4-ol (**4**, 9.7%) and α-terpineol (**5**, 3%). Furthermore, sesquiterpenes like caryophyllene (**6**), valencene (**7**) and germacrene-D (**8**) form an essential part of the overall flavor.

Fig. 3. The hairy fruits of *Delpydora macrophylla* Pierre containing five seeds.

Fig. 4. Fruits of *Dacryodes klaineana* H. J. Lam.

Some green, slightly fermented fruits lying on the ground drew our attention with their strong tropical aroma. They belonged to medium-sized trees of the middle layer of the rainforest, from which we could collect 500 g of intact fruits. They were grape-sized, dark green with a bright yellow fruit flesh inside. The surface was covered with a resinous liquid. A strong passion fruit aroma with a woody impact emerged from the flesh, which was modified by grape, mango and strawberry notes. The plant was identified as *Garcinia epunctata* Stapf, which belongs to the Clusiaceae (Mangosteen family). Analysis of an extract indicated that caryophyllene is mainly responsible for the woody aroma. This compound made up 22.6% of all volatiles. Furthermore, other sesquiterpenes amounted to 37% of all volatiles. Some unspecific estery notes were caused by methyl and ethyl butanoate; however the principle that caused the passion fruit aspect could not be identified directly. Applying GC-olfactometry we detected a sulfurous, blackcurrant-like smelling compound, which we identified after separation on silica gel as 3-sulfhydrylbutanoic acid methyl ester. To the best of our knowledge, this compound has not been described as occurring in nature before. On a chiral column we achieved a separation of the *R* and *S* isomers [12]. Integration of the relevant signals indicated that there is a high enantiomeric excess of 96.6% of the *R*-isomer (Fig. 6). The biochemistry which leads to this compound is not yet known.

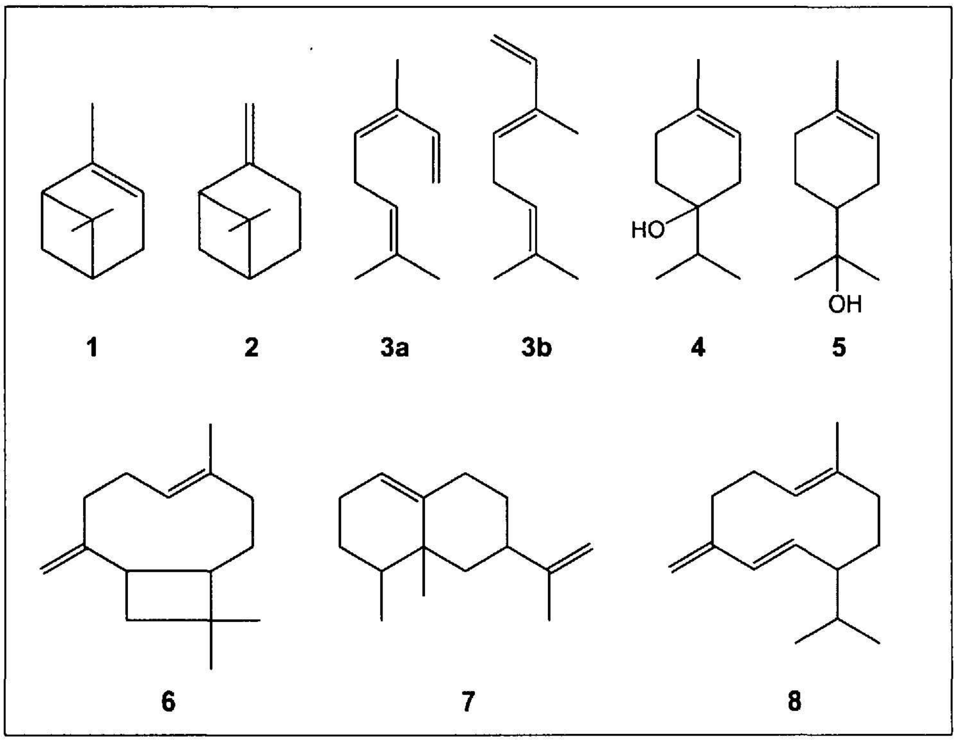

Fig. 5. Characteristic terpenes of the fruits of *Dacryodes klaineana* H. J. Lam.

The exceptional opportunity on this mission was the large dirigible balloon. A ride with it on top of the canopy allows access to fruits normally not reachable. Gliding over the vast green ocean we detected large, orange- and green-colored fruits (Fig. 7) growing on lianas, which were identified as *Landolphia owariensis* P. Beauv. from the Apocynaceae family. Inside a thick wall we found large dark brown seeds, surrounded by a delicious sweet, succulent, white fruit pulp with a strong, fresh, exotic, fruity, creamy, floral flavor reminiscent of cherimoya, apricot and pineapple in yogurt. In the camp we isolated the volatile compounds from the fruit pulp by the headspace method described. The main character impact molecules are listed in Table 1: Linalool, linalooloxide and α-terpineol contribute to the fruity floral part; methyl 2-hexenoate and methyl hexanoate impart a very refreshing exotic fruity top note.

The ethnobotanists made us aware of the bark of a 'garlic tree', *Scorodophloeus zenkeri* Harms. It is a huge tree which can reach up to the canopy top and belongs to the Fabaceae family. The local people use the bark as it is or roasted. When we tore off some bark, a very intense garlic-like odor covered the entire surroundings. A wood tasting like garlic is something quite unusual, but seems to be not so uncommon to the tropics. Beside this species, we found two additional 'garlic trees' with similar aroma properties: *Afrostyrax kamerunensis* Perkins & Gilg (Styracaceae) and *Hua gabonii* de Wild. (Celastraceae).

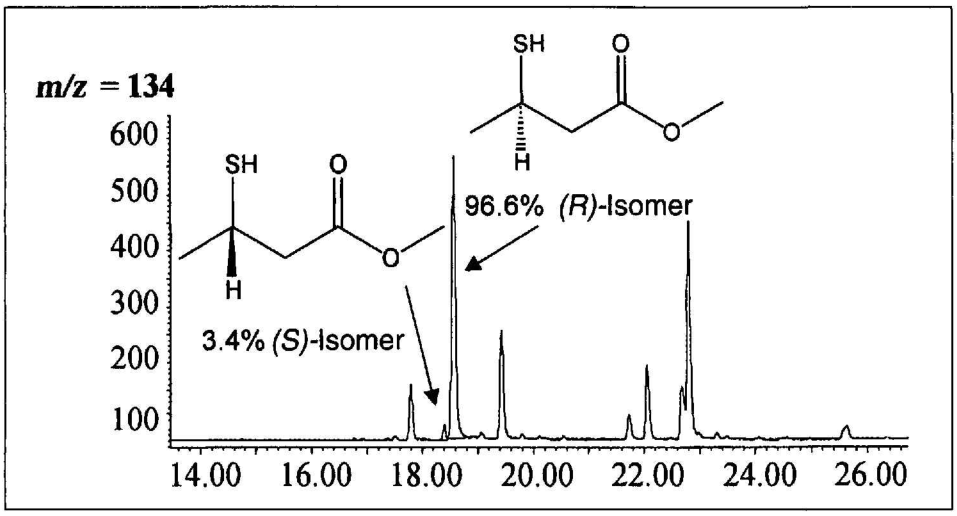

Fig. 6. Separation of *(R)*- and *(S)*-3-sulfhydryl butanoic acid methyl ester on a Cyclodex B column (J&W); detection by GC-SIM-MS; temperature programm: 35 °C for 2 min, with 3 °C/min to 230 °C.

Fig. 7. Fruits of the liana *Landolphia owariensis* P. Beauv.

Table 1. Main character impact compounds of *Landolphia owariensis* P. Beauv., collected Feb. 8th 1999, forêt des Abeilles, Camp Makandé, Gabon.

Compound	Area [%]
3-methyl-1-butanol	2.33
methyl hexanoate	14.20
(E)-2-heptenal	2.46
methyl *(E)*-2-hexenoate	10.40
octanal	0.65
(E)-2-octenal	0.69
cis-linalool oxide	18.10
methyl benzoate	0.26
trans-linalool oxide	5.48
linalool	9.25
methyl salicylate	1.70
α-terpineol	3.56

For a comparative study, 'garlic' barks were collected from small trees of the three species. The headspace aroma was collected on Tenax TA and brought back to our laboratory for analysis. As expected from their sensory properties many sulfur compounds were identified [13]. The three species have some compounds in common, but differ significantly in the ratio of these. The most important compounds are displayed in Table 2, and their structures are given in Fig. 8. Dimethyl disulfide was found as a major compound in all samples, and it was also the major component in the headspace of *H. gabonii* de Wild. 2,4,5,7-Tetrathiaoctane (**15**) possesses strong antifungal activity [14]. We assume that the generation of these sulfur compounds helps to protect the plants against microbiological diseases, which is an evolutionary advantage over competing plants.

However, the biochemical pathway by which the sulfur compounds mentioned in Table 2 are formed, is not quite clear. It is well known that cystein sulfoxide lyases occur in plants *e.g.* in Brassicaceae [15] and Alliaceae [16]. Chin and Lindsay [17] explain the formation of dimethyl disulfide (see **19** in the Scheme, R = CH_3) *via* disproportionation of methyl methylthiosulfinate **18**, which is formed from (*S*)-methylcystein sulfoxide **16** by cystein sulfoxide lyase *via* the unstable methylsulfenic acid **17**. The formation of compounds **12** and **15** can hardly be explained from this precursor. It is most likely that they are formed from uncommon amino acids like *(S)*-[(methylthio)methyl]-L-cystein, its sulfoxide or 3-[(methylthio)methylsulfinyl]-L-alanin, which Kubota *et al.* [18] identified in the fruits of *Scorodocarpus borneensis* Becc. (Olacaceae) *via* the pathway depicted in the Scheme (R = CH_2-S-CH_3). They showed that the first one releases **12** and **15** when treated with a cystein sulfoxide lyase. Obviously the sulfur chemistry in trees is more complex than we expected so far, and their biochemistry differs from what we know from Alliaceae and Brassicaceae.

Outlook

During the mission to the tropical rain forest in Gabon we experienced new flavors with uncommon and exiting compositions. Several new aroma compounds were found, which could become valuable for creative aromas, but at the same time these findings point to unknown biochemical pathways, which also inspire further investigation. Our findings were a mere drop in the ocean. The more we go back to conducting sincere field research, the more we realize that nature is our greatest resource and our greatest source of inspiration.

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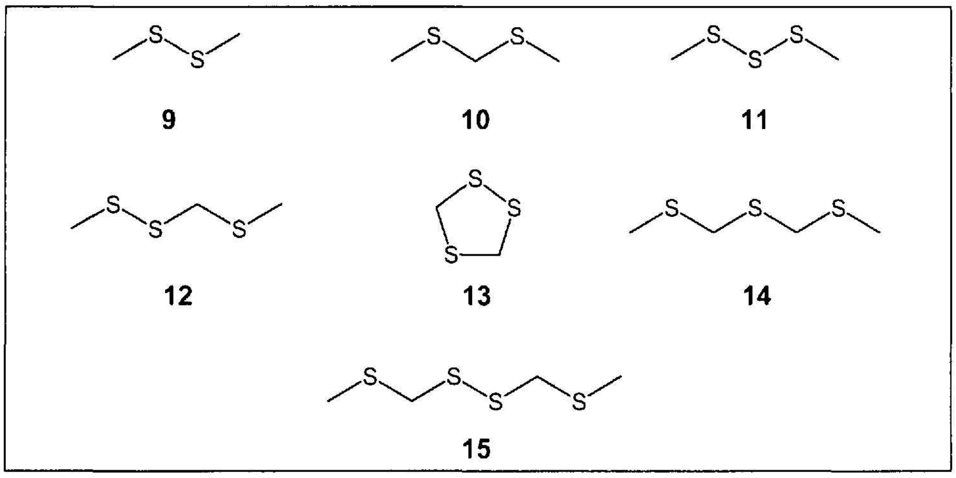


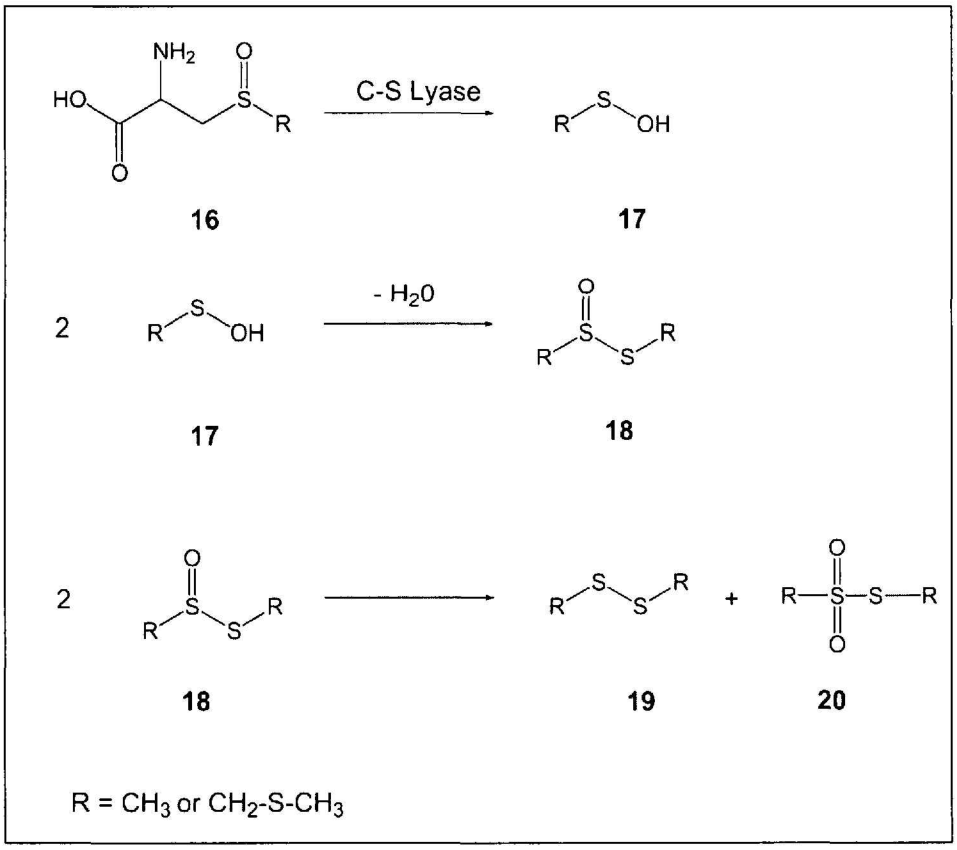
Fig. 8. Sulfur-containing compounds found in the barks of *A. kamerunensis* Perkins & Gilg, *S. zenkeri* Harms. and *H. gabonii* de Wild.

Table 2. Some sulfur-containing compounds found in *A. kamerunensis* Perkins & Gilg, *S. zenkeri* Harms. and *H. gabonii* de Wild., Headspace on Tenax, Mar 3rd 1999, forêt des Abeilles, Camp Makandé, Gabon.

Name	Area% *A. kamerunensis*	*S. zenkeri*	*H. gabonii*
methanethiol	n.d.[a]	n.d.[a]	[b]
dimethyl disulfide (9)	12.65	3.435	78.35
2,4-dithiapentane (10)	3.161	15.470	0.495
dimethyl trisulfide (11)	0.804	n.d.[a]	3.451
2,3,5-trithiahexane (12)	7.252	8.310	8.054
1,2,4-trithiolane (13)	1.051	2.140	0.769
2,4,6-trithiaheptane (14)	0.975	1.496	0.168
2,4,5,7-tetrathiaoctane (15)	1.347	n.d.[a]	n.d.[a]

[a] n.d. = not detected

[b] part of the huge dimethyl disulfide peak



Scheme. Proposed biosynthetic pathway of dimethyl disulfide **9** (R = CH_3) and 2,4,5,7-tetrathiaoctane **15** (R = CH_2-S-CH_3).

[1] F. Hallé, 'Pro-Natura International and Opération Canopée: Biologie d'une canopée de forêt èquatorial IV – Rapport de la mission du radeau des cimes à la Makandé, forêt des Abeilles, Gabon', Pro-Natura International and Opération Canopée, Paris, **1999**, ISBN 2-951 2510-1-7.
[2] H. Stone, J. L. Sidel, in 'Sensory evaluation practice', Academic Press, San Diego, USA, **1993**, 212.
[3] H. Guth, A. M. Murgoci, in 'Flavour Perception Aroma Evaluation', Ed. H. P. Kruse, M. Rothe, Eigenverlag Universität Potsdam, **1997**, p. 233 ff.
[4] C. Masanetz, W. Grosch, *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 114.
[5] R. Teranishi, S. Kint, in 'Flavour Science: Sensible Principles and Techniques', Eds. T. E. Acree, R. Teranishi, American Chemical Society, Washington DC, **1993**, p. 137.
[6] C. Bicchi, D. Joulain, *Flavour Frag. J.* **1990**, *5*, 131.
[7] J.T. Knudsen, L. Tollsten, G. Bergström, *Phytochemistry* **1993**, *33*, 253.
[8] R. Kaiser, L. Tollsten, *Flavour Frag. J.* **1995**, *10*, 153–164.
[9] J.S. Elmore, M.A. Erbahadir, D.S. Mottram, *J. Agric. Food Chem.* **1997**, *45*, 2638.
[10] A. Sen, G. Laskawy, P. Schieberle, W. Grosch, *J. Agric. Food Chem.* **1991**, *39*, 757.
[11] S. Widder, A. Sen, W. Grosch, *Z. Lebensm. Unters. Forsch.* **1991**, *193*, 32.
[12] K. Gassenmeier, W. Grab, X. Yang, R. Eilerman, J. Peppet, in 'Flavour 2000, Proceedings of the 6th Wartburg Aroma Symposium', in press.
[13] X. Yang, D. Josephson, J. Peppet, R. Eilerman, W. Grab, K. Gassenmeier, in '10th International Flavour Symposium Book', E. M. Rothe, Eigenverlag, M. Rothe, Bergholz-Rehbrücke, Germany, **2001**, 145.
[14] K. Kubota, S. Ohhira, A. Kobayashi, *Biosci. Biotech. Biochem.* **1994**, *58*(4), 644.
[15] M. Mazelis, *Phytochemistry* **1963**, *2*,15.
[16] A. Stoll, E. Seebeck, *Adv. Enzymol.* **1951**, *11*, 377.
[17] H.W. Chin, C. Lindsay, *J. Agric. Food Chem.* **1994**, *42*, 1529.
[18] K. Kubota, H. Hirayama, Y. Sato, A. Kobayashi, F. Sugawara, *Phytochemistry* **1998**, *49*(1), 99.