Chimia 54 (2000) 346–363

ISSN 0009–4293

Scents from Rain Forests

Roman Kaiser*

Abstract: Since the dawn of time, the secretive and fragile kingdom of the Rain Forest, which covers 11–13% of all emerged land and shelters eight to nine tenths of all forms of known and unknown life, has harboured extraordinary biological treasures that deserve to be treated with the utmost care. According to a further estimate, 70–80% of these species, *i.e.* around 60% of the entire biodiversity, is located for ecological reasons in the canopy region of the Rain Forests.
Convinced that this richness would also be reflected on the olfactory level, for the past years we have searched in the understory as well as in the canopy of Rain Forests for new attractive scents. This communication opens the discussion of chemical, biological and olfactory aspects of such scents.

Keywords: Atlantic Rain Forest · Canopy · Compositions · Lower Amazonia · Natural scents · Neotropics · Technical infrastructure · Understory

Background and Methods

Up to the middle of the 19th century, natural extracts of scented flowers or other plant parts, and to a certain extent animal secretions, were the only raw materials for the creation of fragrances. No wonder that chemists working in the fragrance industry have investigated these natural products extensively since the dawn of modern organic chemistry. As a result of this (continuing) research work, the perfumer now has at his disposal not only the 500 or so regularly used natural products for the preparation of his creations but at least double this number of synthetic fragrance compounds, which originate from natural products.

In spite of all these synthetic and natural products available from the shelf, the huge range of fascinating natural scents surrounding us is still a great source of stimulation, and far from exhausted. For a long time, however, many of these scents could not be analytically explored because they could not be captured in sufficient amounts and/or in adequate quality.

By the 1970s, methods of instrumental analysis – particularly capillary gas chromatography and mass spectrometry – had reached such a high level of sensitivity that the scents given off by the flower, plant or fruit, – their so-called headspace – could be captured directly and the resulting samples subjected to analytical investigations. In the mid 1970s we at Givaudan thus implemented a long-term research program with the aim of investigating, and subsequently reconstituting synthetically these original and attractive scents, which are not available as an essential oil or related product from the perfumers' shelf. Papers published by Mookherjee *et al.*, Joulain, Tsuneya *et al.*, Kamaki, Brunke *et al.*, Nakamura *et al.*, Surburg *et al.*, and others show that other companies within the fragrance industry probably started to address this topic at about the same time.

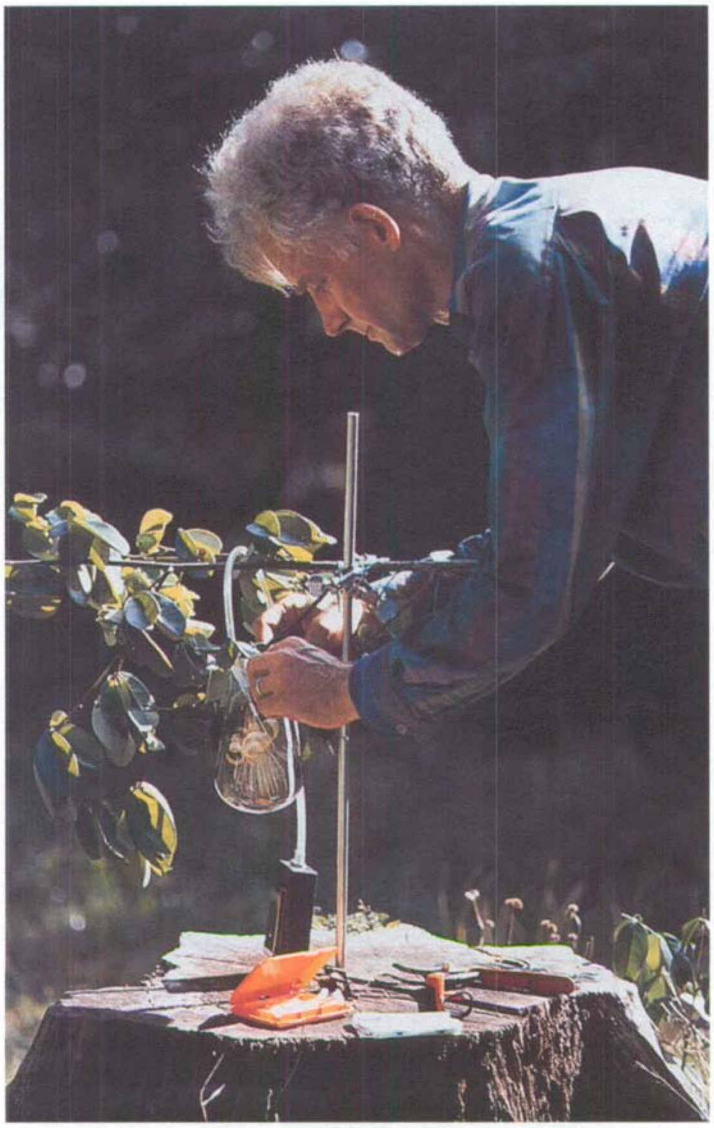
Before such investigations can be carried out, a sufficient quantity of the flower/plant scent – if possible 10 to 50 μg – must be available. This is approximately the amount given off by a moderately fragrant flower over the course of one hour. To capture such a scent sample, various methods can be applied which are compiled in recent review papers by Bicchi and Joulain [1], Kaiser [2], Dobson [3], and Knudsen, Tollsten and Bergström [4]. Since these individual approaches cannot be discussed in more detail on this occasion, I would like to summarise briefly the method I have applied since the 70s, which is the trapping of the emanated scent on a small amount of a suitable adsorbent, such as a porous polymer like Porapak or Tenax or charcoal, followed by solvent extraction. The method has since proved to be effective, and has been specially adapted over the past ten years for field experiments under extreme conditions.

To collect the flower scent of *e.g. Pachira insignis*, a fascinating Bombacaceae native to the neotropics, a single flower is placed in a glass vessel of adapted size and shape without damaging the flower.

The scented air surrounding the flower is now drawn through the adsorption trap by means of a battery-operated pump over a period of 30 min to 2 h (30 ml/min) depending on the intensity of the scent. The adsorption trap containing 2 to 5 mg of adsorbent, in our case Porapak, is placed as closely as possible to the scent source within the glass vessel. While air and moisture pass unhindered through these micro-traps, the scent is adsorbed and accumulates to amounts of 1–100 μg during this time. For flowers or plant parts with very complex shapes, it is more practical to simply isolate the scent source from the environment as much as

*Correspondence: Dr. R. Kaiser
Givaudan Dübendorf AG
Fragrance Research
CH-8600 Dübendorf
Tel.: +41 1 824 23 55
Fax: +41 1 824 29 26
E-Mail: roman.kaiser@givaudan.com

CHIMIA 2000, 54, No. 6



Roman Kaiser setting up a trapping experiment on the scent of *Pachira insignis*, a Bombacaceae native to Amazonia

Trapping scent samples in the field (*Pachira insignis*)

possible by a suitably shaped object, *e.g.* a glass funnel. The adsorption trap is then centred as near as possible to the position where the scent release is at its maximum. Afterwards, the adsorbed scent is eluted with an adequate amount of a suitable solvent, in our case 20–60 µl of hexane:acetone 10:1, directly into a micro-ampoule which is then sealed and kept cool until the return to the laboratory. Finally, the samples thus obtained are investigated by the combination of capillary gas chromatography and mass spectrometry and complementary methods.

The complementary investigation of a micro-extract of the fragrant plant tissue in question – if available at all for this purpose – is always helpful since it facilitates the estimation of the quantitative data and allows a better insight into the less volatile part of the natural scent. Such micro-extracts may be obtained either by extracting the flowers/plants with highly purified solvents such as hexane in the classical way or with liquid carbon dioxide in modern computer-controlled highly convenient lab systems.

By applying these methods over the past 20 years, we have investigated more than 1300 flower, plant, fruit, wood or herb scents and the results of these investigations have been the basis for many close-to-nature reconstitutions, new perfumery accords or have been directly integrated in the daily compository work. Publications *e.g.* on the scent of orchids [5][6], on the scent of cacti [7] and more generally on new or uncommon volatile compounds in the most diverse floral scents [8][9] give a partial illustration of these investigations.

As illustrated by another publication [10] our approach to trapping, investigating and reconstructing natural scents is, however, not only designed to study well-defined flower, herb, fruit, wood scents; it can also be applied to the investigation of entire olfactory 'scenarios', perceived in a certain environment as *e.g.* in a 'Maquis' biotope at the Ligurian coast [10]. Thus, our hope was not only to encounter interesting new flower/plant scents during our missions in the Atlantic Rain Forest and in Lower Amazonia but also to learn as much as possible about entire environmental scent combinations typical for the neotropics.

CHIMIA 2000, 54, No. 6

Introduction to the Nature of Rain Forests

There are no other places with so much light, warmth and humidity as in West Africa, South East Asia, on the Western Pacific Islands and in South America, from South Mexico across the Amazonian Basin down to South Brazil. As a result of the prevailing climatic conditions: at least 2000 mm of precipitation evenly spread over the year, an average humidity between 75–80% and an annual average temperature between 26–27 °C, the most diverse vegetation has developed in these regions forming, as a whole, the Rain Forest. In total it covers today around 11–13% of the emerged land and hosts, according to a rough estimation, 80–90% of all species of plants, mushrooms and animals (Fig. 1). In Amazonia, for example, more than 100 big tree species may be encountered per hectare which house an enormous number of other plant species and animals. The majority of all these species, according to another estimation 70–80% (corresponding to at least 60% of the entire biodiversity), are living in the canopy region of the Rain Forest, which was until recently nearly inaccessible and was one of the least explored biospheres. All these aspects made it most likely that the canopy would also be an olfactory treasury.

During the past 10 years, modern technical systems have been developed to gain direct access to the canopy, the fulfilment of many botanists' and biologists' dreams. As I will illustrate during the second part of this paper, one of the most efficient systems was available to us to search the canopy of Lower Amazonia for new scents.

From various scent-oriented expeditions to tropical regions – we call them 'ScentTreks'– I would like to discuss in the following some parts of our studies in the Atlantic Rain Forest in the region of Morato, near Guaraqueçaba, in the Brazilian State of Paránâ, and in the Rain Forest of Lower Amazonia in French Guyana.

Scents from the Atlantic Rain Forest

At the time Brazil was discovered, the Atlantic Rain Forest stretched along the entire eastern coast, from the states of Rio Grande do Norte to Rio Grande do Sul, corresponding to 12% of Brazilian territory. Today, it is one of the world's most threatened ecosystems with only 4% of the original forest cover (Fig. 2).

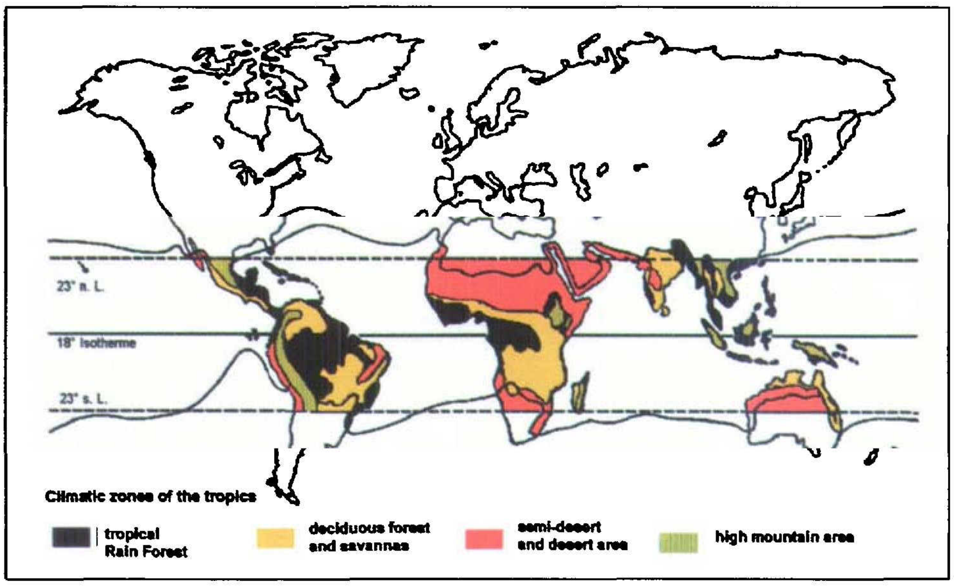


Fig. 1. Climatic zones of the tropics

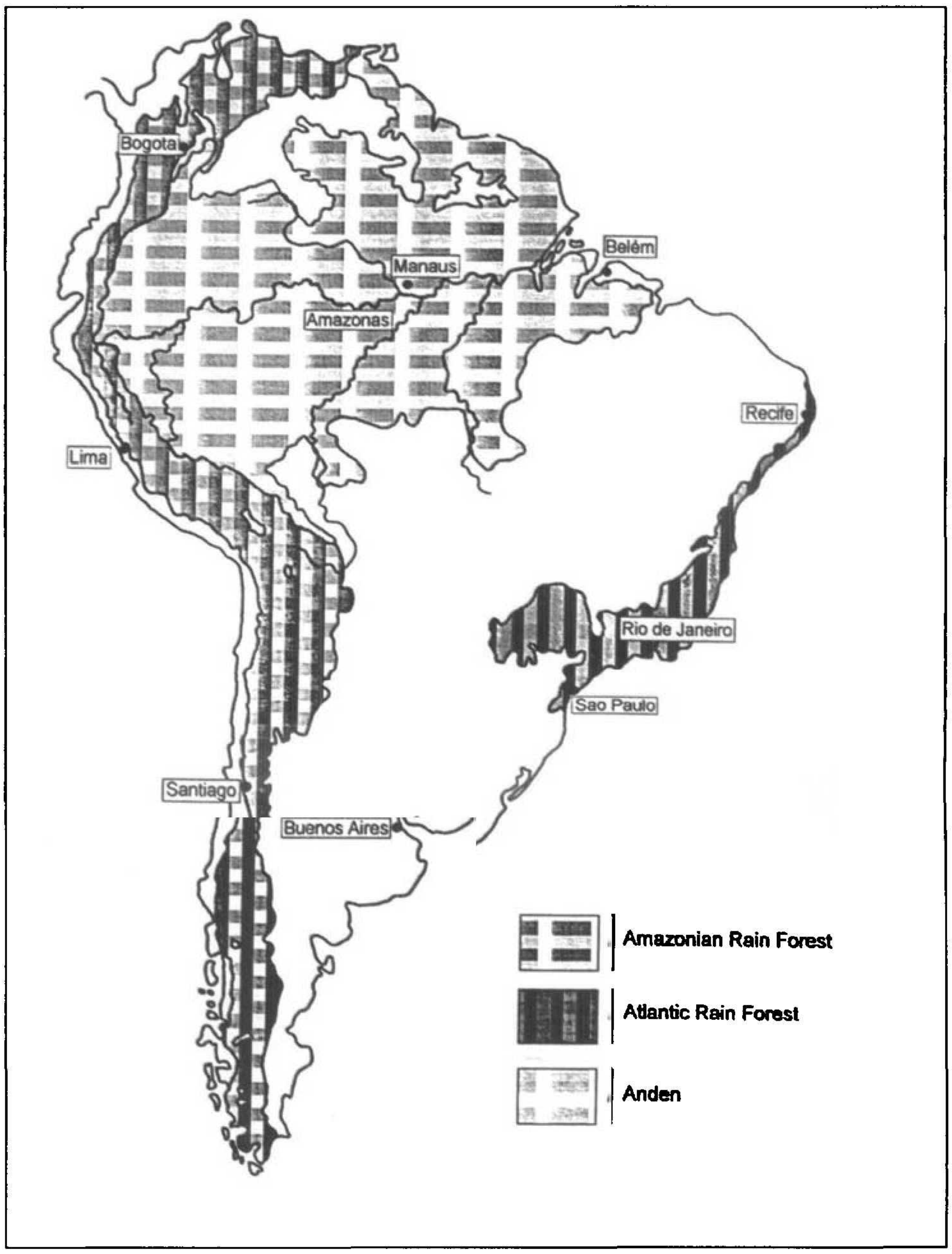


Fig. 2. Rain Forests of South America

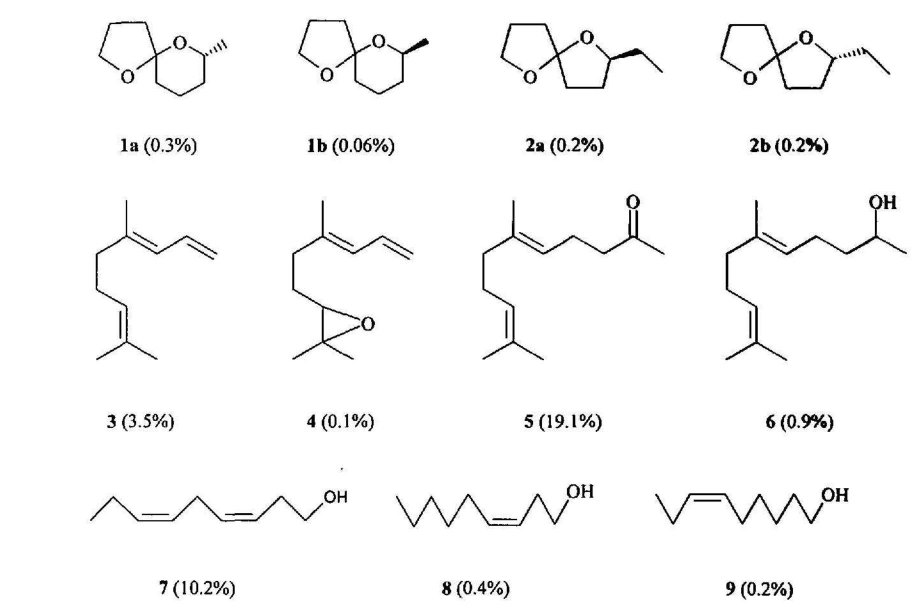
CHIMIA 2000, 54, No. 6

A highly scented biotope along the Morato river in the Atlantic Rain Forest

Fortunately, the 1988 Brazilian Constitution declared it to be a part of the National Heritage and the Foundation of the Atlantic Rain Forest Consortium has in the meantime also gained vital support from Brazilian private companies.

The visited region of Morato, near Guaraqueçaba, is characterised by a rather low average annual temperature of 22 °C, but on the other hand, a very high average relative humidity of over 80%. These conditions favour the growth of an enormous number of epiphytes. Especially the trees at the border to open land are often so heavily covered with species of Gesneriaceae, Bromeliaceae, Araceae, Orchidaceae and even Cactaceae, especially *Rhipsalis* and *Epiphyllum* species, that branches occasionally break. It is not only very humid; water is everywhere with creeks flowing down the slopes and entering the mangrove belt and with impressive falls as at Salto Morato.

The ground vegetation is tighter here than in Amazonia, consisting *e.g.* of large stands of *Heliconias* and various understory palms. At transitions to open land or wherever light comes to the ground vari-



Main Components: Limonene 12.8%, β-Phellandrene 22.2%, Linalool 1%, β-Bisabolene 9,1%, 3,5-Dimethoxytoluene 0.4%, Indole 0.2 %

trapped on Porapak, 10.45-17.00, Sept. 9, 1996, Salto Morato

Fig. 3. Floral scent of *Solanum variabile* (Solanaceae)

CHIMIA 2000, 54, No. 6

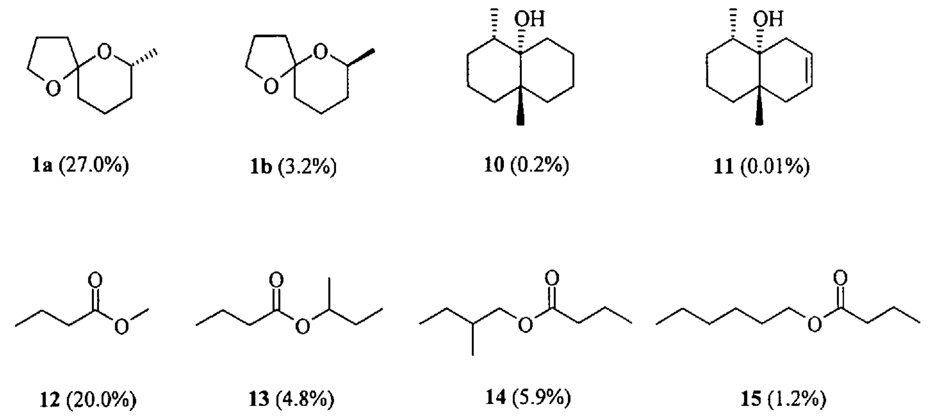
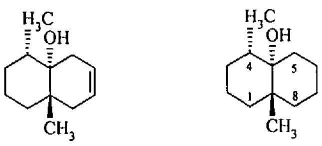


Fig. 4. Floral scent of *Dorstenia turnerifolia* (Moraceae)



		11 (+)-Dehydrogeosmin [%]	10 (-)-Geosmin [%]
Rebutia marsoneri	Werd.	33.0	0.01
Rebutia narvaecense	(Card.) Donald	26.5	-
Echinopsis coronata	Card.	15.0	-
Sulcorebutia kruegeri	(Card.) Ritt.	7.6	-
Echinopsis mamillosa	Gurke. var. *tamboensis*	6.0	-
Dolichothele longimamma	Br. et R.	5.5	-
Pereskia aculeata	Mill.	0.05	1.3
Dorstenia turneraefolia	Fisch. et Mey.	0.01	0.2

Fig. 5. Occurrence of dehydrogeosmin and geosmin

ous Solanaceae can be found. During my visit in early spring, in the second part of September, *Solanum variabile* Mart., the so-called Jurubeba, was the most abundant species in bloom. Their white flowers look similar to those of *Solanum tuberosum* (potato) and they emit a very characteristic melony, watery scent, accompanied by rosy and aromatic-floral notes.

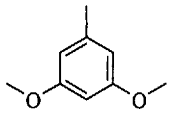
(Z,Z)-3,6-Nonadienol (**7**), accompanied by its dihydro derivatives **8** and **9**, and (*E*)-geranylacetone (**5**) together with the corresponding alcohol **6** (Fig. 3), is largely responsible for these attributes. The occurrence of the epoxide **4** is also remarkable, which is derived from the C_{11} homoterpene **3**, the latter occurring in an extremely large number of natural scents and, in certain species, involved in highly fascinating biological systems of communication [11]. Also the spiroketals **1a/b** and **2a/b** are known to be of semiochemical importance. The mixture of the latter two isomers is known under the name chalcogran as a part of the aggregation pheromone of the bark beetle [12] while **1a/b** have been found by Franke and coauthor [13] in female workers of *Paravespula vulgaris*. These compounds have been identified by us as minor constituents in various flower scents [14] but it is quite remarkable to note that the (*E*)- and (*Z*)-7-methyl-1,6-dioxaspiro-[4.5]decanes (**1a/b**) could even be found as dominating constituents. Thus, they represent around 10% (*E*:*Z* ~ 9:1) of the floral scent of a *Dorstenia* species (Moraceae) occurring in the Morato region. In the Amazonian *Dorstenia turnerifolia* Fisch. & Mey. **1a/b** occur to even around 30% (Fig. 4). Olfactorily, this scent is strongly dominated by geosmin (**10**), which is accompanied by a trace of dehydrogeosmin (**11**). Among a large number of plant species analytically investigated in our laboratory as well as in others [15], geosmin (**10**) and dehydrogeosmin (**11**) were only identified in a series of *Cactaceae* species and, as an interesting exception, in *Dorstenia turnerifolia* [16] (Fig. 5). Geosmin creates, together with the esters **12–15**, an extremely unusual musty-fruity scent.

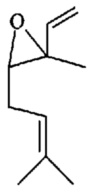
Acnistus arborescens (L.) Schlecht. – the so-called 'Maria Mole' – is another Solanaceae typical to the Southern Atlantic Rain Forest which is in bloom during early spring. This species occurs frequently together with *Solanum variabile*, is of shrub-like to tree-like growth habit and develops a great number of white flowers along twigs and branches which are reminiscent of a *Cestrum* species. They emit a characteristic sweet aromatic floral scent, which is based on an accord generated by 3,5-dimethoxytoluene (**16**), jasmone, anisaldehyde and methyl anthranilate (Fig. 6).

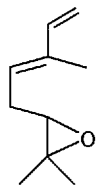
The most characteristic early spring scent in the Guaraqueçaba area has its origin, however, in blooming trees of *Inga marginata* Will. (Leguminosae). The bottle-brush-like white inflorescences covering these trees emit an extremely diffusive fruity-floral scent which is characterised by a strong blackcurrant note, the latter showing a tendency to cat urine, if the nose is too close. This note could be localised by sniffing at the exit of the GC and, after pre-concentration of the corresponding region, a mass spectrum could be recorded which was identical to that of 4-mercapto-4-methylpentan-2-one (**20**), the adduct of hydrogen sulfide to mesityl oxide (**19**) (Fig. 7). This mercapto-ketone shows an extremely low perception threshold of around 0.05 ng/l air and has been described as an aroma impact product of sauvignon wines [17]. Furthermore, **20** has been identified as one of the many compounds formed during Maillard reactions involving cysteine, ribose and phospholipid [18].

CHIMIA 2000, 54, No. 6

Main Components	Area [%]*)
Limonene	2.9
β-Phellandrene	4.6
(*Z*)-Ocimene	1.2
(*E*)-Ocimene	46.0
Linalool	1.0
Methyl benzoate	1.6
3,5-Dimethoxytoluene	32.0
Jasmone	1.1
Anisaldehyde	0.2
Methyl anthranilate	0.2


16 (32.0%)


17 (0.03%)

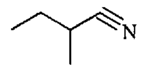

18 (0.03%)

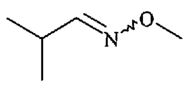
*) trapped on Porapak, 09.50-14.00, Sept. 23, 1996, Salto Morato

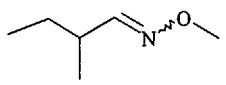
Fig. 6. Floral scent of *Acnistus arborescens*, the so-called 'Maria Mole'

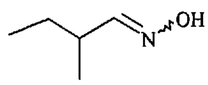
Main Components	Area [%]*)
(*E*)-Ocimene	17.0
trans-Linalool oxide (furanoid)	5.8
2,6,6-Trimethyl-2-vinyl-THP-5-one	2.6
cis-Linalool oxide (furanoid)	2.9
Linalool	6.9
Caryophyllene	47.2
Humulene	3.0
Linalool oxides (pyranoid)	7.0
2,6-Dimethyl-3,7-octadiene-2,6-diol	0.9
Caryophyllene epoxide	0.3


19 (0.2%) **20** (0.00001%)


21 (0.05%)

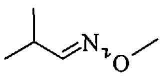
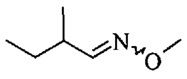
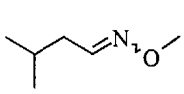

22 (0.2%)


23 (0.2%)


24 (0.3%)

*) trapped on Porapak, 15.00-19.00, Sept. 25, 1996, Salto Morato

Fig. 7. Floral scent of *Inga marginata*, the so-called 'Inga Feijão'

		22	23	25
		[%]	[%]	[%]
Carissa macrocarpa	Apocynaceae	-	0.6	-
Cerbera odollam	Apocynaceae	-	0.2	-
Coffea arabica	Rubiaceae	-	1.5	-
Curcumis melo	Curcurbitaceae	-	0.2	0.05
Dioclea reflexa	Papilionaceae	-	1.1	-
Inga marginata	Leguminosae	0.2	0.2	-
Hedychium gardnerianum	Zingiberaceae	-	-	0.07
Lonicera caprifolium	Caprifoliaceae	-	-	0.02
Nicotiana alata	Solanaceae	-	0.03	0.4
Nicotiana suaveolens	Solanaceae	-	-	0.2
Posoqueria latifolia	Rubiaceae	-	0.1	-
Salix caprea	Salicaceae	-	0.6	-
Tabernaemonta ventricosa	Apocynaceae	-	0.08	-

Fig. 8. Occurrence of the oxime-O-methyl ethers **22**, **23**, and **25** in natural scents

CHIMIA 2000, 54, No. 6

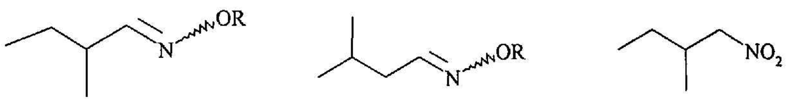
During the past few years **20** has proved to be rather widespread in nature and is found as a very significant olfactory trace constituent in many additional natural scents or flavours in box tree, Japanese green tea, grapefruit, basil leaves, tomato leaves and *Peaonia lutea*.

In the scent of *Inga marginata*, **20** is accompanied by the structurally and olfactorily interesting isobutyraldoxime-O-methyl ether (**22**) and 2-methylbutyraldoxime-O-methyl ether (**23**) (*E*:*Z* for **22** and **23** ~ 15:1).

Over the last 15 years the oximes of isobutyraldehyde, 2-methylbutyraldehyde (**24**), and isovaleraldehyde (**26**) have been identified in a great variety of natural scents [15]. In contrast, the oxime ethers are less common as natural products. Thus, the methyl ether **22** was so far only detected in the headspace above some microorganisms [19] and the two methyl ethers **23** and **25** have been recently described as occurring in the headspace volatiles of spider mite-infested cucumber leaves [20]. Undamaged leaves do not produce these oxime ethers.

As illustrated by Fig. 8 we could identify these very volatile components in a whole series of floral scents [21]. They occur, for example, together with high amounts of the corresponding oximes **24** and **26** in the white-floral scent of *Posoqueria latifolia*, a Rubiaceae occurring in Central America as well as in the southern part of Brazil (Fig. 9). This small tree, growing not higher than 7 m, has its habitat preferentially along river banks in low lands. The inflorescences terminate the branches and consist of a cluster of white flowers with extremely long corolla tubes, well adapted to pollination by hawk moths. In fact the flowers begin to open an hour before dusk, and shortly thereafter they fill the night with a very enjoyable intense spicy white-floral scent.

Main Components	Area [%]*)
(*E*)-Ocimene	2.2
1-Nitro-2-methylbutane (**27**)	0.8
2-Methylbutyraldoxime (**24**)	9.0
Isovaleraldoxime (**26**)	10.1
Methyl benzoate	6.7
Germacrene D	34.1
Benzyl acetate	1.5
Methyl salicylate	1.0
Benzyl alcohol	0.6
Benzyl tiglate	0.9
Eugenol	1.2
Benzyl salicylate	2.5



23 R = CH_3 (0.1%) **24** R = H (9.0%)
25 R = CH_3 (0.1%) **26** R = H (10.1%)
27 (0.8 %)

1a (0.1%) **2a/b** (0.2%, 1:1)

*) trapped on Porapak, 15.00-20.00, June 3, 1994, Bogor

Fig. 9. Floral scent of *Posoqueria latifolia*

The world's largest dirigible is used to gain access to the canopy

Scents from the Rain Forest of Lower Amazonia

In a unique project organised by Pro-Natura International, a well-known non-profit Rain Forest preservation group, we had the opportunity to search in the canopy as well as in the understory of the Amazonian Rain Forest of French Guyana for new natural scents. The project was divided into two missions: the first mission took place from September 30 to October 16, 1996 in the region of Camp Parakou, 30 km west of Kourou; the second mission was from November 29 to

CHIMIA 2000, 54, No. 6

December 11, 1996 in the region of Camp Voltaire, 80 km south of Saint Laurent du Maroni.

The technical infrastructure to access the canopy consisted of a dirigible of 50 m x 20 m holding 8500 m^3 air and additionally equipped with a diesel engine for the fine approach, which is designed to carry either

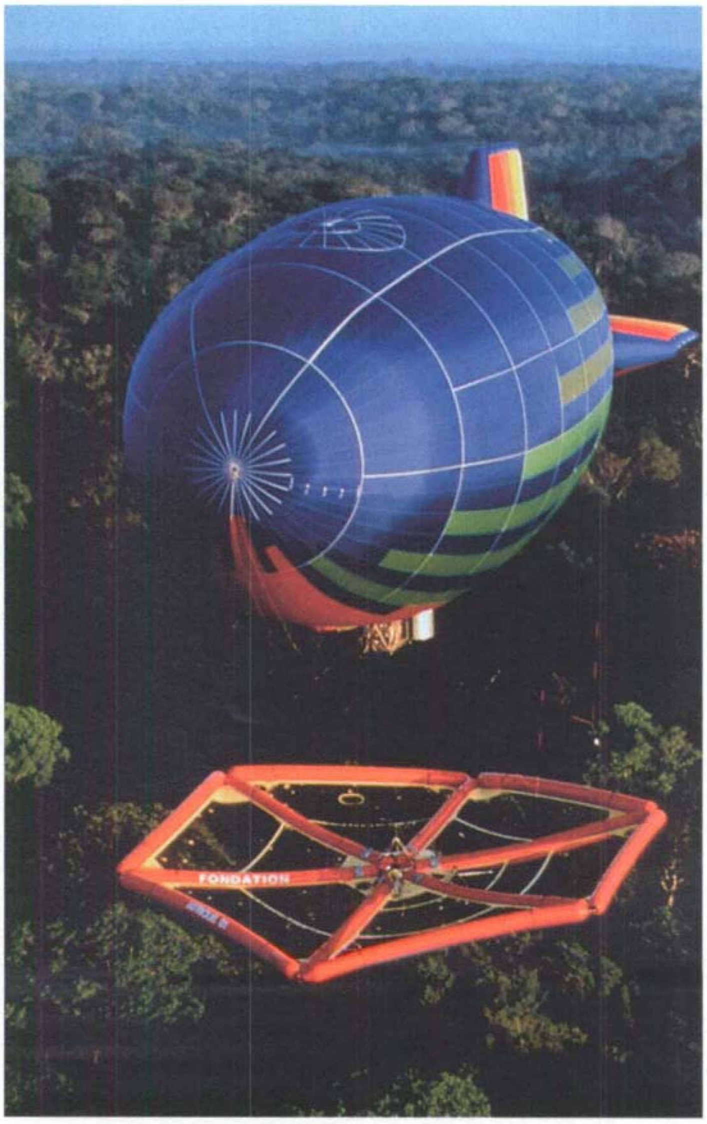
- a raft (the so-called radeau des cimes) of hexagon shape measuring 600 m^2 to be placed on the canopy for stationary studies over a longer period of time

or

- a so-called skimmer or sledge (triangle with sides of 5.5 m) with up to three researchers on it being directly brought to the trees of interest.

This highly efficient system was developed and is operated under the guidance of Francis Hallé, professor for tropical botany at the University of Montpellier, Dany Cleyet-Marrel, designer and pilot of the dirigible, and Gilles Ebersolt, inventor and designer of the raft. More technical information is summarised in the Mission Reports [22].

One of our first discoveries in the canopy of Parakou was *Macoubea guianensis* Aubl., a rather frequent Apocynaceae in Lower Amazonia, which blooms in September and October with clusters of white star-like flowers similar to those of *Tabernaemontana* species. In the late evening through to the early morning,



The dirigible carrying a raft of 600 m^2 which can be placed on tree tops for stationary studies over a longer period of time

The dirigible carrying the so-called skimmer with up to three researchers on it

CHIMIA 2000, 54, No. 6

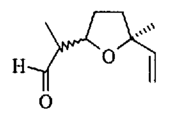
flowering trees are perceivable from far by their very extraordinary scent of yoghurt and milk turned bad, calamus, algae and 'white flowers'. Linalool and the linalool oxides, accompanied by the structurally related isomers **29a–d** of lilac alcohol and **28a–d** of lilac aldehyde, represent the main components of this scent (Fig. 10). Much more relevant to the olfactory characteristics are, however, the uncommon C_{10}-lipid metabolites **30–33**. The dienal **30** and the corresponding alcohol **32** were also recently found in the floral scent of *Brassavola flagillaris* [21], an orchid native to Minas Gerais in Brazil, where they are the dominating constituents not only regarding their olfactory contribution but also regarding quantity (Fig. 11).

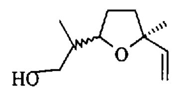
The (*Z*,*Z*)-4,7-decadienal (**30**) has been described as a minor compound with character impact in the oil of *Acorus calamus* [23] and the (*E*,*Z*,*Z*)-2,4,7-decatrienal as an off-flavour of strongly autoxidised oils [24] and other food as well as a lipoxygenase cleavage product in freshwater diatoms (*Melosira varians*) [25].

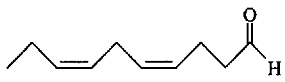
On the other side we found in the most unusual scent of the Venezuelan orchid *Rodriguezia refracta* Rchb. f. [14b] around 85% of (*E*,*Z*)-2,4-decadienal (**34**), accompanied by 2.4% of its precursor (*E*,*Z*,*Z*)-2,4,7-decatrienal (**31**) contributing a typical codliver aspect and the corresponding alcohol **33**, which is also present in *Macoubea guianensis* (Fig. 10). It may be assumed that the C_{10}-lipid metabolites of all these floral scents are formed from linolenic acid *via* the common intermediate (*E*,*Z*,*Z*)-2,4,7-decatrienal (**31**) (Fig. 12). Preferred reduction of the C(7)-(8)-double bond leads to the typical constituents of *Rodriguezia refracta*, that of the C(2)-(3)-double bond to those of *Brassavola flagillaris* and *Macoubea guianensis*, respectively. The structural proof for **31** was obtained by synthesis following in principle the procedure described by Meijboom and Stroink [24] by coupling 1-bromo-2-pentyne with (*E*)-2-penten-4-ynol and subsequent selective oxidation and hydrogenation using Lindlar's catalyst (Scheme 1). Reduction of **31** with lithium aluminium hydride finally gave the trienol **33**, which was not yet known as a natural product.

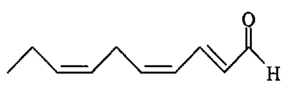
Some other highly interesting lipid metabolites could be identified in the floral scent of *Coryanthes elegantium* Linden Rchb. f., a rare epiphyte native to the hot and humid Rain Forests of Western Ecuador and Western Colombia. Besides the olfactorily dominating (*E*,*Z*)-

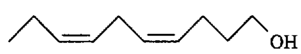
Main Components	Area [%]*)
Sabinene	4.3
1,8-Cineole	8.2
trans-Linalool oxide (furanoid)	21.1
cis-Linalool oxide (furanoid)	1.4
α-Cubebene	2.4
Linalool	31.1
Germacrene D	6.4
trans-Linalool oxide (pyranoid)	7.2
Benzyl alcohol	2.9

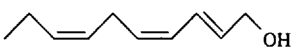

28a-d (0.3%)


29a-d (0.25%)


30 (10.0%)


31 (0.3%)


32 (0.9%)


33 (0.1%)

*) trapped on Porapak, 10.00-14.00, Oct. 10, 1996, Camp Parakou

Fig. 10. Floral scent of *Macoubea guianensis*

C_{10}-Lipid Metabolites in *Brassavola flagillaris* (Orchidacea)

(*Z*,*Z*)-4,7-Decadienal (**30**)	2.6%
(*Z*)-4-Decenol	0.2%
(*Z*)-7-Decenol	0.3%
(*E*,*Z*,*Z*)-2,4,7-Decatrienal (**31**)	0.05%
(*Z*,*Z*)-4,7-Decadienol (**32**)	18.4%
Decan-4-olide	0.1%
(*Z*)-7-Decen-4-olide	0.2%

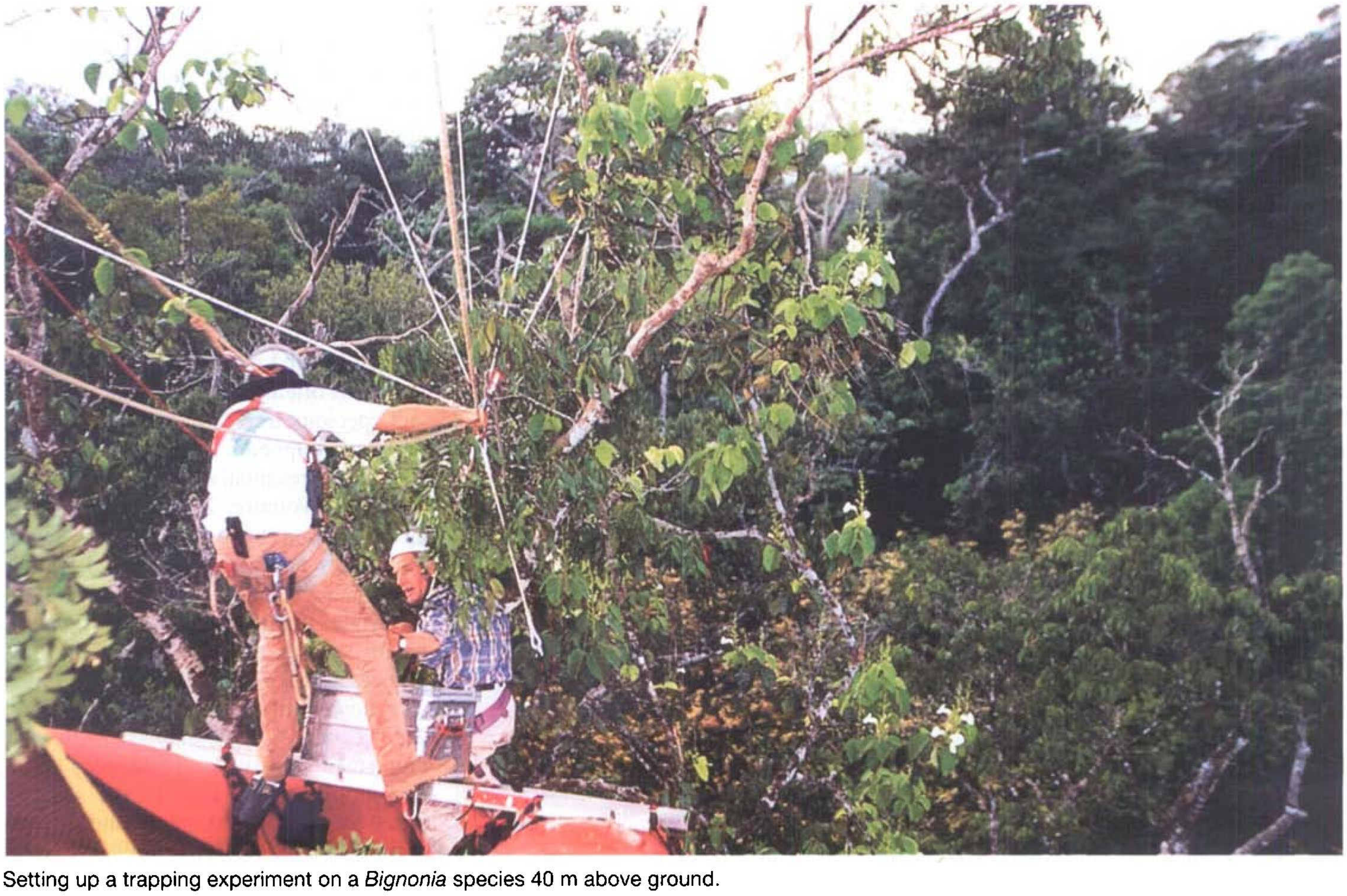
C_{10}-Lipid Metabolites in *Rodriquezia refracta* (Orchidacea)

(*E*,*Z*)-2,4-Decadienal (**34**)	85.0%
(*E*,*E*)-2,4-Decadienal	3.7%
(*E*,*Z*,*Z*)-2,4,7-Decatrienal (**31**)	2.4%
(*E*,*Z*)-2,4-Decadienol	2.5%
(*E*,*E*)-2,4-Decadienol	0.1%
(*E*,*Z*,*Z*)-2,4,7-Decatrienol (**33**)	0.03%

Fig. 11. C_{10}-Lipid metabolites in *Brassavola flagillaris* and *Rodriguezia refracta*

CHIMIA 2000, 54, No. 6

Looking down to the highly diverse tree composition of the canopy (Camp Voltaire, French Guyana).



Setting up a trapping experiment on a *Bignonia* species 40 m above ground.

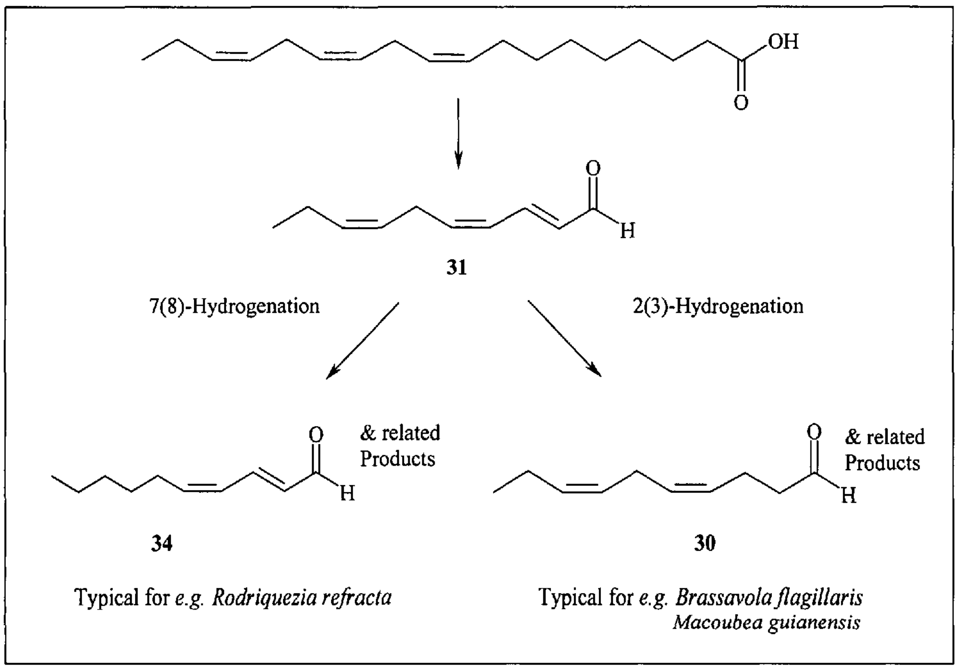
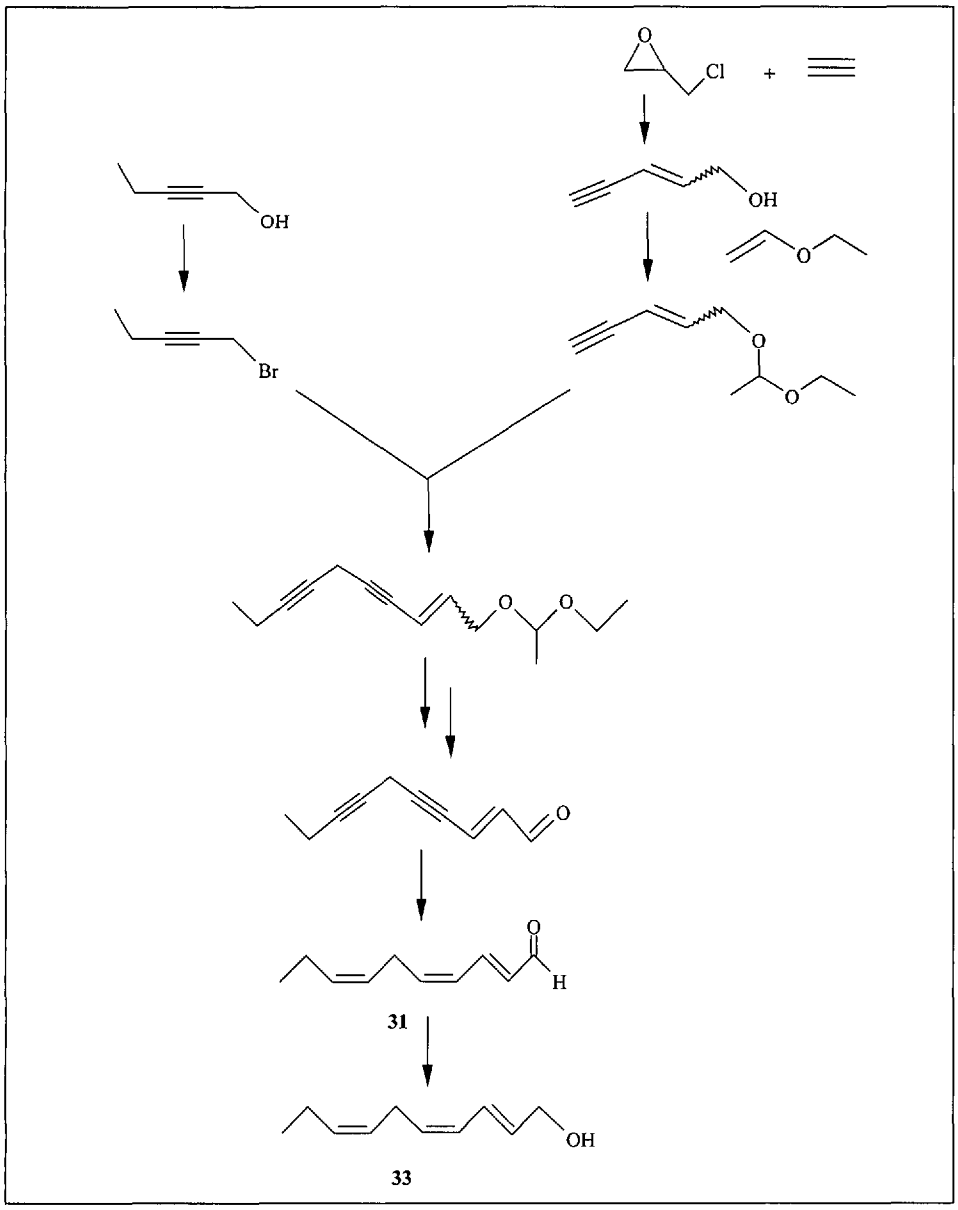


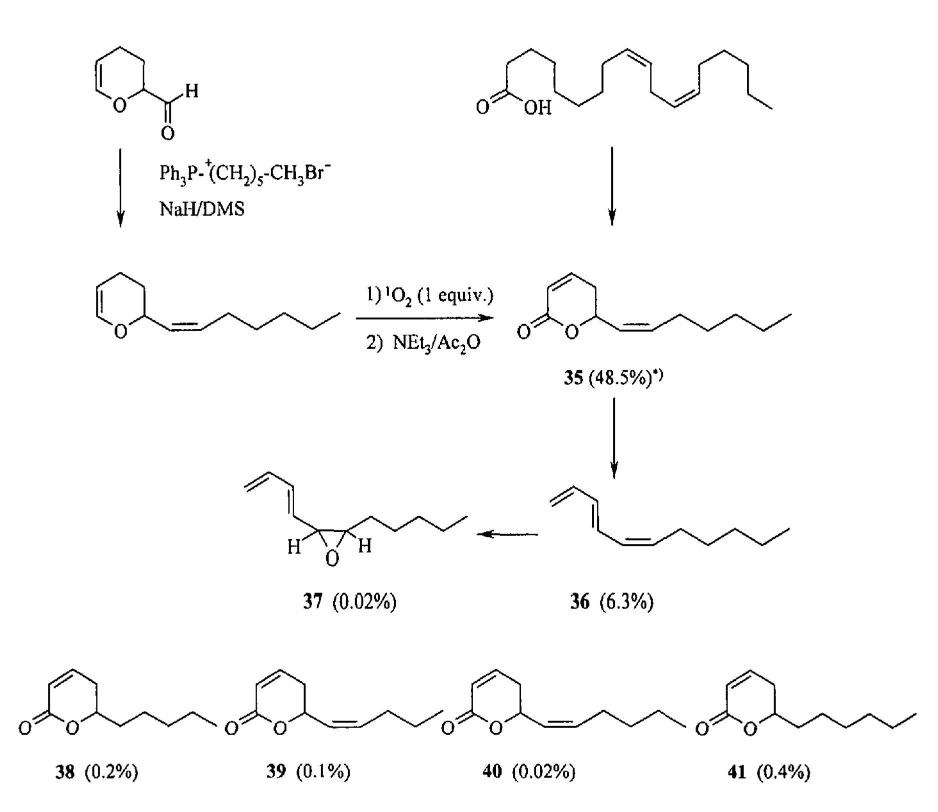
Fig. 12. (*E,Z,Z*)-2,4,7-Decatrienal, the intermediate of many C_{10}-lipid metabolites



Scheme 1. Synthesis of (*E,Z,Z*)-2,4,7-decatrienal (**31**)

1,3,5-undecatriene (**36**), the trapped scent consisted to around 50% of an oxygen-containing compound of molecular weight 194 showing a much longer retention time (Scheme 2). Based on its mass fragmentation, a 4- or 5-dodecadienolide could be assumed. Fortunately, the absolute amount of the trapping enabled 20 mg of the compound to be isolated by preparative capillary gas chromatography and its structure was elucidated by NMR as (*Z*)-2,6-dodecadien-5-olide (**35**). It is apparent from Scheme 2 that **35** has to be the biological precursor of the (*E,Z*)-1,3,5-undecatriene (**36**) which is present in high isomeric purity. This is in contrast to galbanum oil which contains the (*E,Z*)- and (*E,E*)-isomers in a ratio of approximately 3:1 [26]. A literature search revealed that **35** was described already in 1977 by Priestap and coauthors [27] as a constituent of the rhizomes of *Aristolochia argentina* and that it was synthesised four years later by Fehr and coauthors [28] together with a series of other 6-substituted 5,6-dihydro-2(2H)-pyranones using the approach outlined in Scheme 2. This possible metabolite of linolic acid is accompanied in the scent of *Coryanthes elegantium* on the level of minor constituents by (*Z*)-5(6)-epoxy-(*E*)-1,3-undecadiene (**37**), massoia lactone (**38**), (*Z*)-2,6-decadien-5-olide (**39**), (*Z*)-2,6-undecadien-5-olide (**40**) and 2-dodecen-5-olide (**41**). The dienolides **39** and **40**, which are not yet known to occur in nature, have been synthesised by applying the same method as for **35**. The epoxide **37**, which is also a new natural product, was obtained by epoxidation of **36**. We found **35** and **36** in a whole series of *Coryanthes* species all belonging to the section *Coryanthes*.

Let us come back to the Amazonian Rain Forest of French Guyana, in which the family of Lecythidaceae (Brazil nut family) is often among the most abundant and specious tree families. Correspondingly, we trapped a respectable series of fragrant representatives at Camp Parokou and Camp Voltaire. The majority of species turned out to be dominated by a single floral scent component (*e.g.* 1,8-cineol, ocimene, linalool, a-farnesene, methyl salicylate, indole; compare [29]), a few, however, give off more equilibrated fragrances as, for example, the most impressive flowers of the famous Cannon Ball Tree (*Couroupita guianensis*), with their unmistakable rosy floral and spicy scent. As summarised in Fig. 13 the scent is based on nerol and neral and related products including derivatives and eugenol and isoeugenol (compare also [30]).



Scheme 2. (*E,Z*)-1,3,5-Undecatriene (**36**) and its precursor **35** in the *Coryanthes* species

Main Components	Area [%]*)
(*E*)-Ocimene	12.0
Linalool	1.0
Neral	1.7
Geranial	1.6
Neryl acetate	13.8
Geranyl acetate	2.6
Nerol	31.8
Geraniol	2.5
Phenylethyl alcohol	2.5
Eugenol	7.5
(*E*)-Isoeugenol	3.5
(*E,E*)-Farnesol	2.5

*) trapped on Porapak, 10.00 - 14.00, Oct. 11, 1996, Camp Parakou

Fig. 13. Floral scent of *Couroupita guianenesis* (Lecythidaceae), the so-called 'Cannon Ball Tree'

Also the yellow flowering *Lecythis* sp. FG 44 (voucher FH 4397), which we trapped at Camp Parakou, shows a more complex scent composition worthy of brief discussion. Olfactorily, this scent is dominated by ionones, especially β-ionone (**44**), which is accompanied by comparatively high amounts of its photocyclisation product **42** and its intermediate, the (*Z*)-retro-γ-ionone (**43**) (Fig. 14).

Correspondingly, caryophyllene (**46**), the main component of *Lecythis* sp. FG 44, seems to undergo partial photoisomerization to isocaryophyllene (**45**) (Fig. 15), a component all too often described as naturally occurring. Among 1300 plant species investigated so far in our laboratory, caryophyllene (**46**) was found in 300, isocaryophyllene (**45**) only in six representatives. Important to the woody aspect of *Lecythis* sp. FG 44 are the caryophyllene epoxides **48** and **49** including the isocaryophyllene epoxide **47** as well as the caryophylladienols **50–53**.

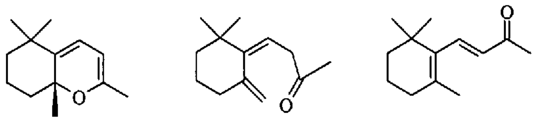
Ocimene and caryophyllene, accompanied by an astonishing high amount of indole, are also the main scent constituents of the magical aquamarine blue *Lecythis persistens* Sagot subsp. *persistens* which we discovered during our mission at Camp Voltaire.

In this scent, the main component, caryophyllene, is accompanied by its epoxide and the caryophyll-5-en-2-α-ol (**55**) (Fig. 16). Up to now we found **55** only in the floral scent of the Colombian orchid *Bollea coelestis* [14c], but in this case it is the main constituent totally characterising its distinct woody fragrance. The tertiary alcohol **35** is formed to about 0.5%, together with other side products, by the oxymercuration/cyclization/reduction of caryophyllene (**46**) to the caryophyllan-2,6-α-oxide (**56**). Selectively, it is accessible from the described diepoxide **58** [31] by reductive cleavage of the 2(12)-epoxy group with lithium aluminium hydride to the epoxy alcohol **59** followed by desepoxidation with Zn/Cu couple (Scheme 3). By contrast, the corresponding epimeric alcohol **62** was found by us in trace amounts in the scent of *Gongora cassidea*, an orchid native to the mountain forests from Mexico to Costa Rica. The tertiary alcohol **62** is easily accessible from caryophyllene (**46**) *via* kobuson (**60**) [32].

Within 30 min hiking distance westward of Camp Voltaire is, surrounded by the intact Rain Forest, a small savannah-like biotope of about 300 x 200 m with an extreme biodiversity. It was generated by a landslide which blocked a creek and now, as a consequence, the entire area

CHIMIA 2000, 54, No. 6

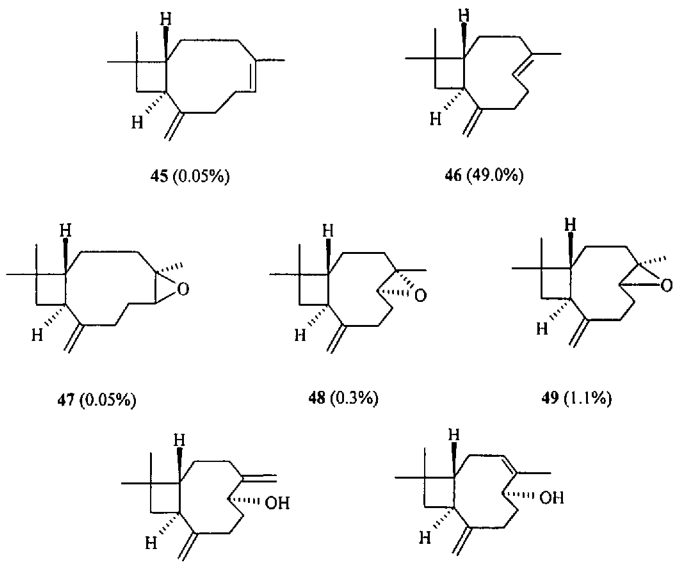
Main Components	Area [%]*)
(*E*)-Ocimene	31.1
Caryophyllene	49.0
Methyl benzoate	1.6
Cyclic-β-ionone (**42**)	1.3
Humulene	0.7
Methyl salicylate	3.8
Dihydro-β-ionone	1.8
β-Ionone (**44**)	1.0
Caryophyllene epoxide	1.1



42 (1.3%) **43** (0.05%) **44** (1.0%)

*) trapped on Porapak, 10.00-15.00, Oct. 15, 1996, Camp Parakou

Fig. 14. Floral scent composition of *Lecythis sp.* FG 44



45 (0.05%) **46** (49.0%)

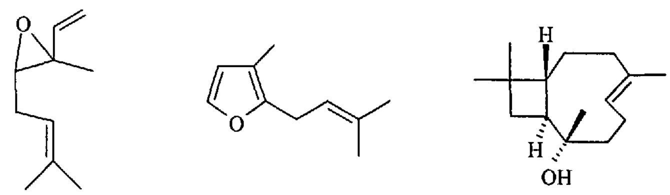
47 (0.05%) **48** (0.3%) **49** (1.1%)

50 β-OH (0.05%) **51** α-OH (0.2%) **52** α-OH (0.1%) **53** β-OH (0.02%)

Fig. 15. Carphyllene derivatives in *Lecythis sp.* FG 44/1

floods during the rainy season. The trees have already partially lost their leaves and are now heavily covered by epiphytes, parasitic plants, vines and lianas, which bloom even near the ground. In this biotope we had the chance to find many spectacular tropical plants in bloom, such as *Philodendron nobile*, a *Clitorea* species with a very delicate creamy vanilla-like scent, *Passiflora maliformis*, emitting an enjoyable aromatic-floral scent elegantly rounded off by a note of tropical fruits, or *Catasetum barbatum*, one of the amazing euglossine orchids, attracting with a balsamic aromatic-floral, indolic fragrance, the respective euglossine bees or *Epidendrum ciliare* growing as big clusters on the trunks of palm trees. This commonly occurring species has spread from Central America down to Amazonia and has, in contrast to many other orchids, adapted itself to widely differing living conditions. As a typical moth orchid it emits its appealing refreshing white-floral scent, which is based on high amounts of linalool, (*E*)-ocimene epoxide (**18**) and 2-methylbutyraldoxime (**24**) (Fig. 17), exclusively during the night. As illustrated by Fig. 18 the absolute amount of scent given off from one flower per hour dramatically increases after sunset, reaching a peak at between 10 pm and

Main Components	Area [%]*)
Myrcene	8.1
(*Z*)-Ocimene	1.7
(*E*)-Ocimene	25.0
(*E*)-3(4)-Epoxy-3,7-dimethyl-1,6-octadiene	0.8
Linalool	19.0
Germacrene D	7.0
Phenylacetonitrile	1.4
Caryophyllene epoxide	0.2
1-Nitro-2-phenylethane	0.2
Indole	21.1

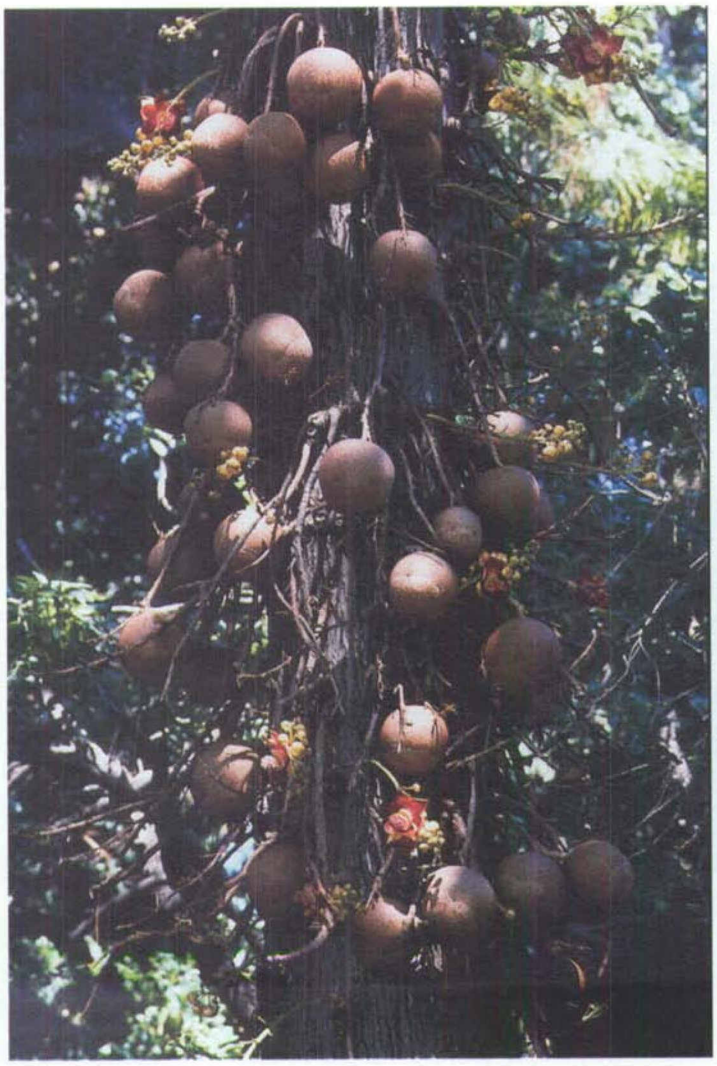


17 (0.8%) **54** (0.1%) **55** (0.1%)

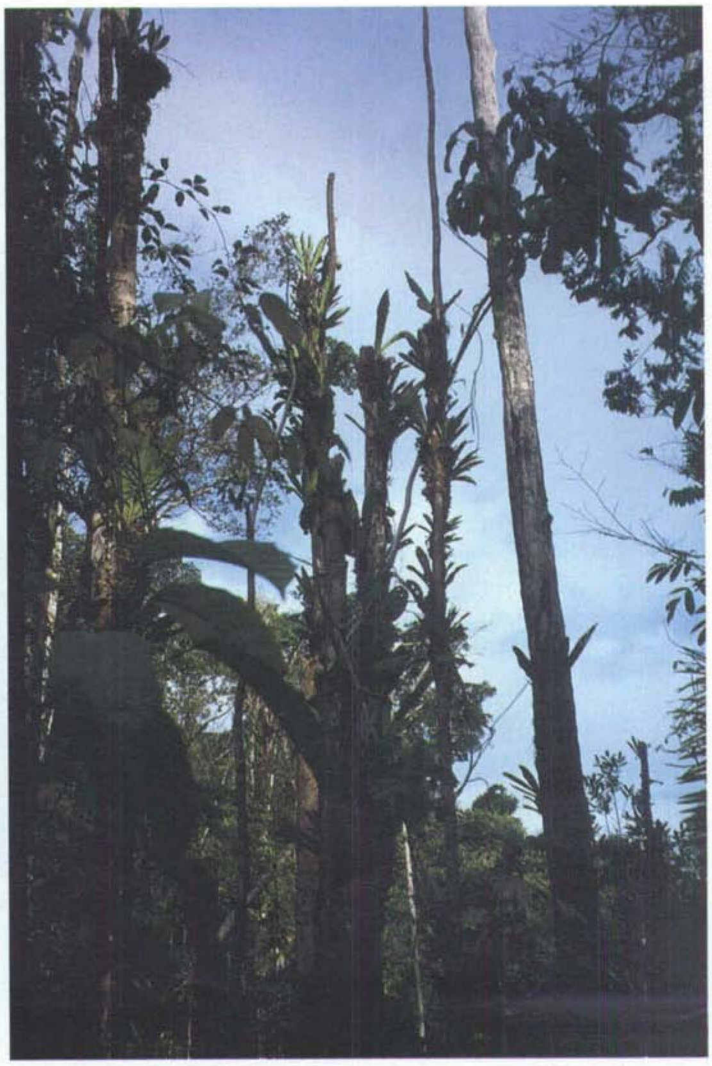
*) trapped on Porapak, 10.00-12.30, Dec.9, 1996, Camp Voltaire

Fig. 16. Floral scent of *Lecythis persistens* Sagot subsp. *persistens*

CHIMIA 2000, 54, No. 6

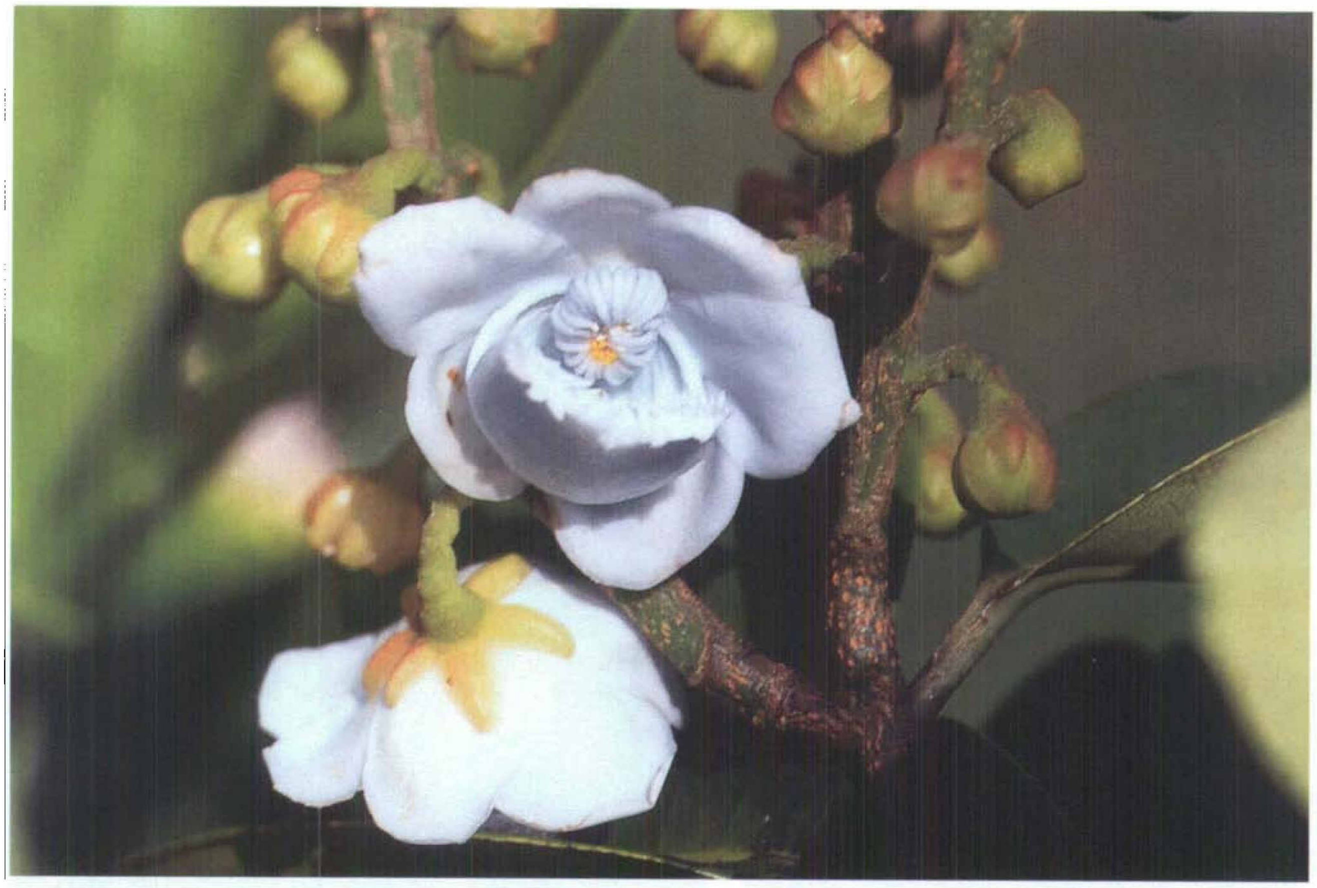


Couroupita guianensis (Lecythid-aceae), the famous Cannon Ball Tree, a native of Lower Amazonia.



A unique biotope full of death and thriving life near Camp Voltaire in French Guyana

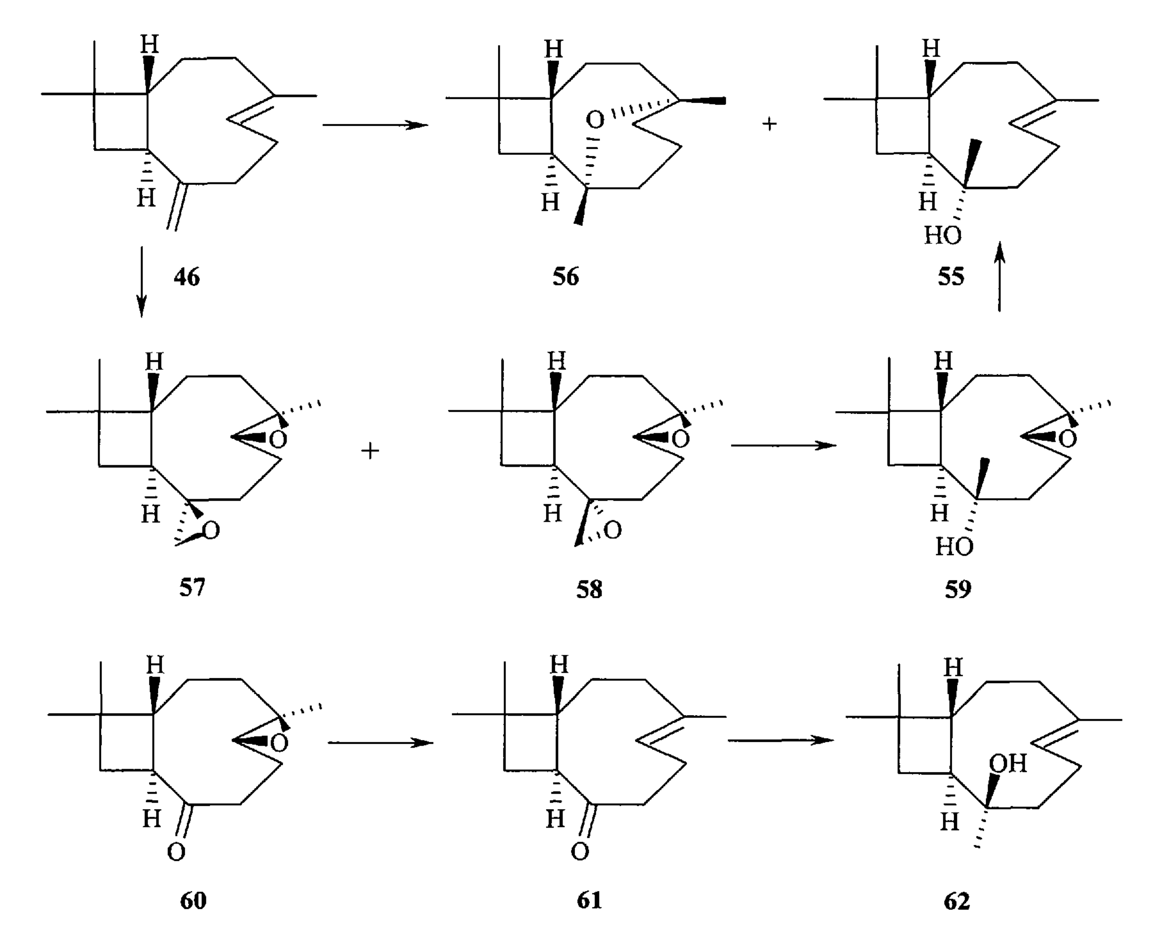
The spectacular flowers of *Couroupita guianensis* emit a characteristic rosy-floral and spicy scent



The magical aquamarine blue flowers of *Lecythis persistens subsp. persistens* are attractive also from a olfactory and chemical point of view

One of the many gems found in the biotope near Camp Voltaire: *Passiflora maliformis*

CHIMIA 2000, 54, No. 6

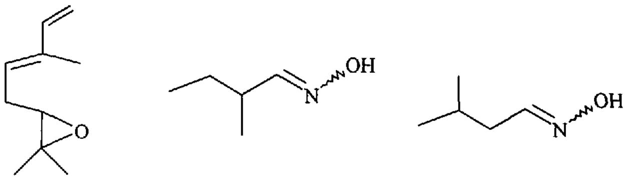


Scheme 3. Caryophyll-5-en-2-α-ol, a minor scent constituent of *Lecythis persistens* Sagot subsp. *persistens*

11 pm, and gradually subsiding during the early morning hours. If the analogous graphs are plotted for the individual scent constituents as illustrated by Fig. 19, it becomes clear that the maxima do not have to coincide. This explains the well-known fact that not only the quantity but also the quality of the scent given off by a flower or plant may be time-dependent, an aspect very important to us in connection with the reconstitution of such scents.

With another highly scented epiphyte typical for such biotopes, with *Cochleostema odoratissima*, I would like to conclude my discussion of some selected scents encountered in neotropic Rain Forests. This Commelinaceae emits a very distinct fragrance, which is composed of rosy-floral, ionone-floral and refreshing hesperidic notes and a high portion of indole. Especially worth mentioning is the occurrence of the (*E*)-2(3)-dihydrofarnesal (**64**) (Fig. 20), which adds an attractive fresh aldehydic 'lily of the valley'-type note to this rich floral scent, and that of the corresponding alcohol **65** rounding it off by a tender note of

Main Components	**Area [%]*)**
(*E*)-Ocimene	8.5
(*E*)-Ocimene epoxide	10.2
2-Methylbutyraldoxime	8.2
Isovaleraldoxime	0.6
Linalool	53.0
Methyl benzoate	1.8
Germacrene D	2.4
(*E*,*E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	6.9



18 (10.2%) **24** (8.2%) **26** (0.6%)

*) trapped on Porapak, 20.00-22.00, Dec. 10, 1996, Camp Voltaire

Fig. 17. Floral scent of *Epidendrum ciliare* (Orchidaceae)

CHIMIA 2000, 54, No. 6

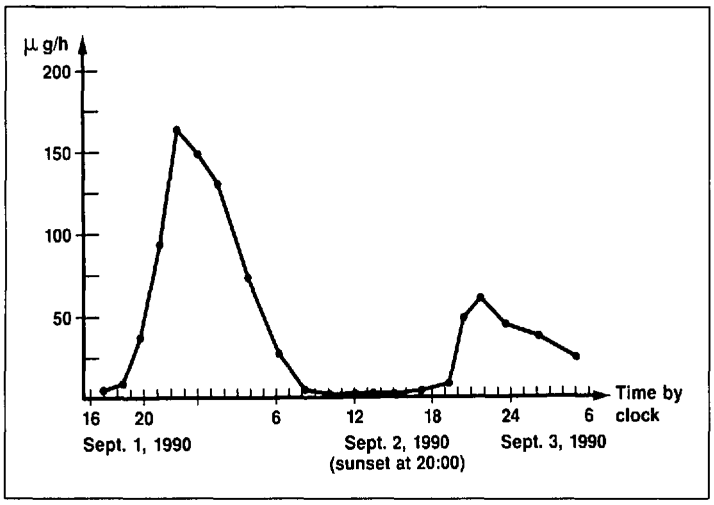


Fig. 18. *Epidendrum ciliare*: emanation of scent in the course of two subsequent nights

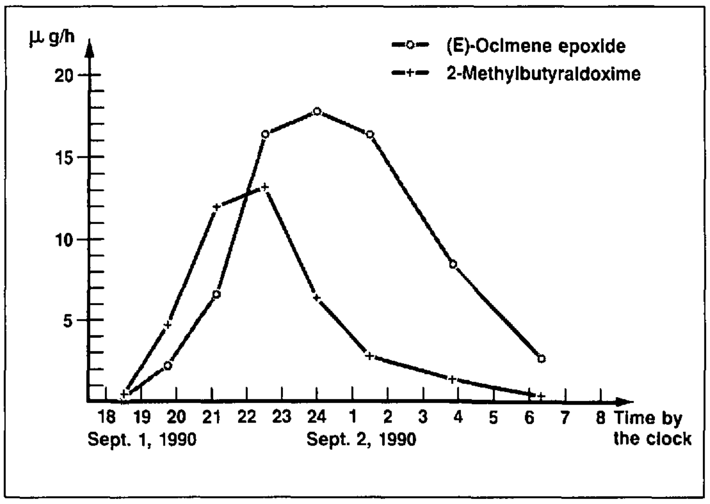
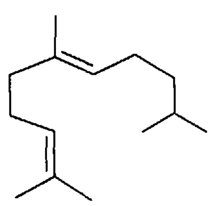
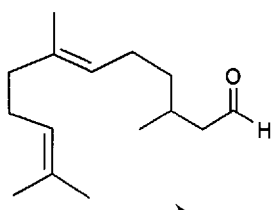


Fig. 19. *Epidendrum ciliare*: changes in the amounts of two scent constituents during one night

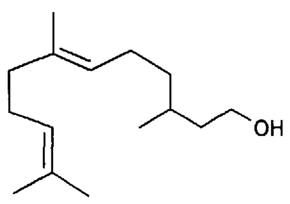
Main Components	Area [%]*)
(*E*)-Ocimene	5.0
6-Methyl-5-hepten-2-one	2.8
Linalool	5.0
Neral	1.0
Geranial	2.0
Citronellol	3.0
Nerol	39.5
Dihydro-β-ionone	2.0
(*E*)-Geranylacetone	6.8
(*E*)-6,10-Dimethyl-5,9-undecadien-2-ol	0.5
(*E*)-2(3)-Dihydrofarnesal (**64**)	1.5
(*E*)-2(3)-Dihydrofarnesol (**65**)	5.3
Indol	11.5



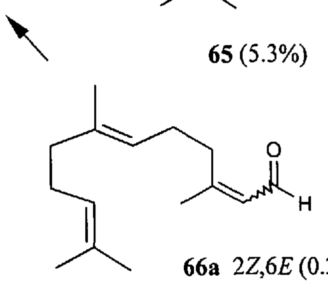
63 (0.03%)



64 (1.5%)



65 (5.3%)



66a 2*Z*,6*E* (0.2%)
66b 2*E*,6*E* (0.3%)

*) trapped on Porapak, 11.00-15.00, Dec. 11, 1996, Camp Voltaire

Fig. 20. Floral scent of *Cochleostema odoratissima* (Commelinaceae)

cyclamen. As we recently described, **64** plays also an important role in the floral scent of *Citrus limon* [10]. This product has found in the meantime its way to the perfumer's shelf [33] and helps to give modern fragrances a unique natural floral freshness.

Acknowledgements

I am grateful to Dr. M. Whitten, Florida Museum of Natural History, Gainesville, FL, for samples of the trapped scent of *Brassavola flagillaris*; Dr. Ch. Weymuth, University of Zürich, for samples of the trapped scent of *Coryanthes elegantium*; to Mr. Miguel Krigsner for his hospitality during my studies in the Morato region; to Prof. Francis Hallé for his help in identifying the plant species encountered in Amazonia; to the entire team of 'Canopy 1996' to make the unique missions in French Guyana possible; to Mr. K. Lombard, Givaudan Teaneck (USA), for his help during the mission at Camp Parakou; to Mr. J. Guichard, Director of Fine Fragrances of Givaudan Paris, for his help during the mission at Camp Voltaire; to my colleagues at Givaudan Research Ltd for discussions and assistance, in particular to Dr. J. Schmid for many discussions on the mass spectra of new components, to Mr. M. Rothaupt and Mr. E. Senn for the isolation of new compounds in the µg range by preparative capillary GC; to Mr. J. Märki for many indepth NMR-studies, to Dr. D. Helmlinger for the synthesis of the (*E,Z,Z*)-2,4,7-decatrienal (**31**), to Ms Aurelia Kreis for all the organisation and secretarial work, to Dr. Katja Schultz for discussions and synthetic help and, last but not least, to Mr. V. Clerc and Mr. M. Jenzer for their skilful assistance throughout the investigations summarised in this paper.

CHIMIA 2000, 54, No. 6

Received: May 15, 2000

[1] C. Bicchi, D. Joulain, 'Headspace gas chromatographic analysis of medicinal and aromatic plants and flowers', *Flavour and Frag. J.* **1990**, *5*, 131–145.
[2] R. Kaiser, 'Trapping, investigation and reconstitution of flower scents', chapter 7 in 'Perfumes: Art, Science and Technology', Eds. P.M. Müller, D. Lamparsky, Elsevier Applied Science, London & New York, **1991**, p. 213–250.
[3] H.E.M. Dobson, 'Analysis of flower and pollen volatiles', in 'Modern Methods of Plant Analysis', New Series, vol. 12, 'Essential Oils and Waxes', Springer, Berlin & Heidelberg, **1991**, p. 231–151.
[4] J.T. Knudsen, L. Tollsten, G. Bergström, 'Floral Scents – A Checklist of Volatile Compounds Isolated by Head-Space Techniques', *Phytochemistry* **1993**, *33*, 253–280.
[5] R. Kaiser, 'On the Scent of Orchids', in 'Bioactive Volatile Compounds from Plants', ACS Symposium series 525, Eds. R. Teranishi, R.G. Buttery, H. Sugisawa, American Chemical Society, Washington, DC, **1993**, p. 203–219.
[6] R. Kaiser, 'The Scent of Orchids', Elsevier, Amsterdam, **1993**.
[7] R. Kaiser, 'An Introduction to the Scent of Cacti', *Flavour Frag. J.* **1995**, *10*, 153–164.
[8] R. Kaiser, 'New or Uncommon Volatile Compounds in Floral Scents', in 'Flavours, Fragrances and Essential Oils', Proc. 13th International Congress of Flavours, Fragrances and Essential Oils, Istanbul, Turkey, 15–19 October 1995, vol 2, AREP. Publ., Istanbul, **1995**, p. 135–168.
[9] R. Kaiser, 'New or uncommon volatile compounds in the most diverse natural scents', presented at 15ème Journées Internationales Huiles Essentielles, Digne-les-Bains, France, 5–7 September 1996, *Rivista Italiana EPPOS* **1997**, 18.
[10] R. Kaiser, 'Environmental Scents at the Ligurian Coast', *Perfume & Flavorist* **1997**, *22*, 7–18.
[11] M. Dicke, P. van Baarlen, R. Wessels, H. Dijkman, 'Herbivory induces systemic production of plant volatiles that attract predators of the herbivore: extraction of endogenous elicitory', *J. Chem. Ecol.* **1993**, *19*, 581–599.
[12] W. Francke, V. Heemann, B. Gerken, J.A.A. Renwick, J.P. Vité, '2-Ethyl-1,6-dioxaspiro[4,4]nonane, principal aggregation pheromone of *Pityogenes chalcographus* (L)', *Naturwiss.* **1997**, *64*, 590–591.
[13] W. Francke, G. Hindorf, W. Reith, 'Alkyl-1,6-dioxaspiro[45]decanes – a new class of pheromones', *Naturwiss.* **1997**, *66*, 618–619.
[14] R. Kaiser, 'The Scent of Orchids', Elsevier, Amsterdam, **1993**, a) 166–167; b) 119; c) 51.
[15] J.T. Knudsen, L. Tollsten, L.G. Bergström, 'Floral Scents – a Check-list of Volatile Compounds Isolated by Headspace Techniques', *Phytochemistry* **1993**, *42*, 253–80.
[16] R. Kaiser, L. Tollsten, 'An Introduction to the Scent of Cacti', *Flavour and Fragrance J.* **1995**, *10*, 153–164.
[17] P. Darriet, T. Tominaga, V. Lavigne, J.-N. Boidron, D. Dubourdien, 'Identification of a Powerful Aromatic Component of *Vitis vinifera* L. var. Sauvignon Wines: 4-mercapro-4-methylpentan-2-one', *Flavour and Fragrance J.* **1995**, *10*, 385–392.
[18] L.J. Farmer, D.S. Mottram, F.B. Whitfield, 'Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid', *J. Sci. Food Argric.* **1989**, *49*, 347–68.
[19] D.B. Harper, J. Nelson, 'The Bacterial Biogenesis of Isobutyraldoxime-O-methyl ether, a Novel Volatile Secondary Metabolite', *J. Gen. Microbiol.* **1982**, *128*, 1667–1678.
[20] J. Takabayashi, M. Dicke, S. Takahashi, M.A.Posthumus, T.A. van Beek, 'Leaf Age Effects Composition of Herbivore-induced Synomones and Attraction of Predatory Mites', *J. Chem. Ecol.* **1994**, *20*, 373–386.
[21] R. Kaiser, 'New or Uncommon Volatile Compounds in Floral Scents', in 'Flavours, Fragrances and Essential Oils', Proc. of the 13th International Congress of Flavours, Fragrances and Essential Oils, Istanbul, Turkey, 15–19 Oct. 1995, vol. 2, 135–168.
[22] a) 'Biologie d'une Canopée de Forêt Equatorial – II. Rapport de Mission: Radeau des Cimes', October, November 1991, Réserve de Campo, Cameroun, eds. Francis Hallé & Olivier Pascal; b) 'Biologie d'une Canopée de Forêt Equatorial – III. Rapport de la Mission d'Exploration Scientifique de la Canopée de Guyane', Octobre – Décembre 1996, eds. Francis Hallé, Pro-Natura International & Opération Canopée.
[23] F.P. van Lier, L.M. van der Linde, A.J.A. van der Weerdt, 'Isolation and Synthesis of (Z,Z)-4,7-Decadienal, the Character Impact Compound in the Oil of *Acorus calamus* L.', in 'Progress in Essential Oil Research', Eds E.-J. Brunke, W. de Gruyter, Berlin, **1986**, 215-225.
[24] P.M. Meijboom, J.B.A. Stroink, '2-*trans*,4-*cis*,7-*cis*-Decatrienal, the Fishy Off-Flavor Occurring in Strongly Autoxidized Oils Containing Linolenic Acid or ω 3,6,9, *etc.*, Fatty Acids', *J. Am. Oil Chem. Soc.* **1972**, *49*, 555–558.
[25] T. Wendel, F. Jüttner, 'Lipoxygenase-mediated Formation of Hydrocarbons and Unsaturated Aldehydes in Freshwater Diatoms', *Phytochemistry* **1996**, *41*, 1445–1449.
[26] B.A. McAndrew, D.M. Michalkiewicz, 'Analysis of Glabanum Oils', in 'Flavors and Fragrances: a World Perspective', Eds. B.M. Lawrence, B.D. Mookherjee, B.J. Willis, Elsevier Science Publishers, Amsterdam, **1998**, 573–585.
[27] H.A. Priestap, J.D. Bonafede, E.A. Ruveda, 'Argentilactone, a Novel 5-Hydroxyacid Lactone from *Aristolochia argentina*', Phytochemistry **1977**, *16*, 1579–1582.
[28] C. Fehr, J. Galindo, G. Ohloff, 'Novel Approach to the Synthesis of 6-substituted 5,6-Dihydro-2(2H)-pyranones', *Helv. Chim. Acta* **1981**, *64*, 1247–1256.
[29] J.T. Knudsen, S.A. Mori, 'Floral Scents and Pollination in Neotropical Lecythidaceae', *Biotropica* **1996**, *28*, 42–60.
[30] A. Bartsch, 'Botanische Kuriositäten aus dem Urwald', *Dragoco Report* **1997**, 63–71.
[31] B. Maurer, A. Hauser, 'New Sesquiterpenoids from Clary Sage Oil', *Helv. Chim. Acta* **1983**, *66*, 2223–2235.
[32] R. Kaiser, D. Lamparsky, 'New Carbonyl Compounds in the High-Boiling Fraction of Lavender Oil', *Helv. Chim. Acta* **1983**, *66*, 1843–1849.
[33] US Patent 5 942 272 (Aug. 24, **1999**) assigned to Givaudan.