PERFUMER & FLAVORIST

Presented at the World Perfumery Congress
May 14-17, 1991, Palma de Mallorca

Natural Isolates from Seville Bitter Orange Tree

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How important may be the scent of the bitter orange flower in the creative imagination and on the compounding palet of the modern perfumer? The fragrance of the rose may be the queen of all odors, but the scent of the bitter orange flower must be the princess of all flower fragrances. In spring the famous bitter orange tree of Seville (introduced by the Arabs in the 11th century) comes into bloom and if you have once smelled the sparkling, youthful, romantic scent of a bitter orange blossom, you will surely never forget it. One can easily understand why young brides wear these flowers at their wedding. In April, we harvest the bitter orange flower for our neroli oil and orange flower water.

Ernest Guenther[1] already stated in 1949 in his well-known encyclopedia on Essential Oils, that:

> "The bulk of Spanish Neroli Bigarade oil has been produced in Seville by a prominent firm, which enjoys a nation-wide reputation for the retail sale of orange flower water."

Now, more than 40 years later, we are proud to state that this reputation has expanded internationally.

The natural isolates, which are produced from the different parts of the bitter orange tree are shown in Scheme 1.

This article deals with the isolates from the flowers, the fruits and the leaves, their isolation method and composition, olfactive properties and ideas for new constituents.

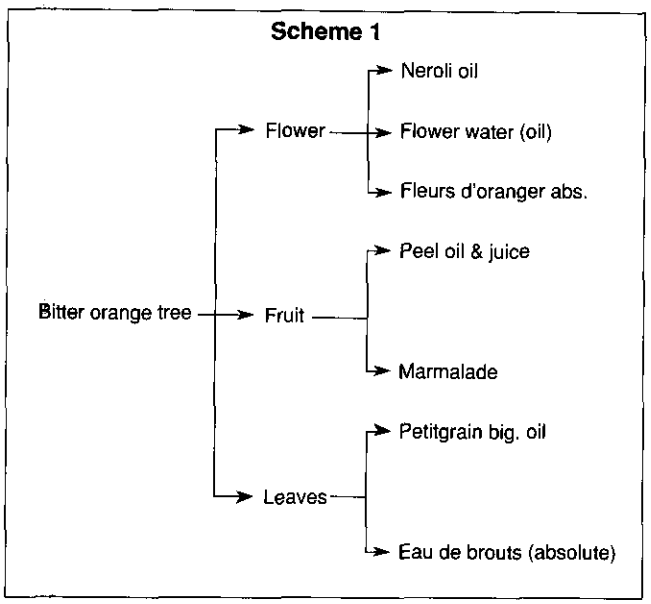
Bitter Orange Flower Derivatives

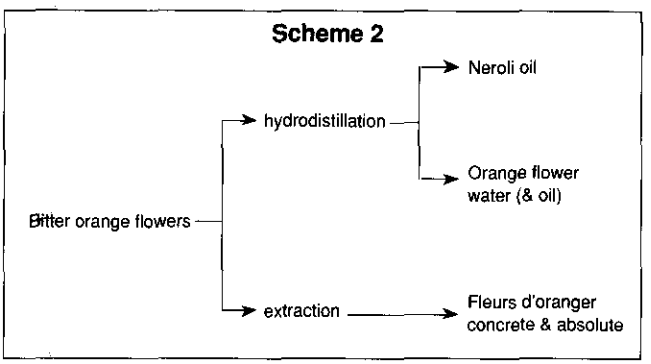
Isolation and Composition of Flower Oils—The naturals are isolated from the flowers by hydrodistillation or by extraction with a suitable solvent (mostly hexane). Hydrodistillation is featured by the fact that the plant

0272-2666/91/0006-0101$04.00/00—

Perfumer & Flavorist (ISSN 0272-2666)
An Allured Publication
Volume 16, Number 6

NOVEMBER/DECEMBER 1991





material (flowers) is heated with a lot of water (2-3 times its weight) and that the steam is generated within the still. Per one kilogram of flowers, 0.5-1 gram of oil and one kilogram of orange flower water is collected. The yield of the oil is seldom higher than 0.1% and more often 0.05 to 0.1%. The orange water may contain another, more soluble, part of the oil in a yield of maximum 0.01% (on fresh plant material).

In the continuous extraction of orange flowers with suitable solvents (hydrocarbons, ethers, ketones) one may obtain 0.2% of an orange flower concrete which, after treatment with ethanol, affords an absolute in 0.1% yield.

Scheme 2 shows the natural isolates from bitter orange flowers.

For the chemical composition of the hydrodistilled oils, it is important to take into account the decomposition of genuine constituents caused by the isolation method under forced conditions, such as high temperature, pH and time. The pH during hydrodistillation may vary from 5-7.

A normal hydrodistilled neroli oil contains at least five times higher concentration of monoterpene hydrocarbons than an extracted absolute. This fact may be an indication that monoterpene hydrocarbons are formed during the isolation of the oil by hydrodistillation, e.g. by decompostion of linalyl acetate. With modern techniques, especially with fused silica capillary columns in GC, one can detect hundreds of constituents in the oils; most of these compounds still unknown or unpublished.

The constituents of the natural isolates from orange flowers can be divided into five main groups:[2]

- monoterpene hydrocarbons (I)
- oxygen-containing monoterpenes (II)
- sesquiterpene hydrocarbons (III)
- oxygen-containing sesquiterpenes (IV)
- aromatic and miscellaneous compounds (V)

Based upon the published data and our own investigations, the various isolates contain the following concentrations:

Group	Hydrodistilled Neroli oil	Hydrodistilled Water oil	Extracted Orange flower absolutes
I	40%	2%	7%
II	50	80	60
III	1	-	1
IV	7	3	15
V	2	15	18

Olfactive Properties of Flower Oils and New Constituents—The olfactive properties of orange blossom and neroli

Table I. Characteristic constituents of white flowers (e.g. bitter orange)

	Sakurai 1979 *C. unshiu*	Kaiser 1980 *C. aurantium*	Joulain 1986 *R.ps. acacia*
benzyl cyanide	4.7%	1.5%	+
methyl anthranilate	2.0	6.5	14.6%
2-aminobenzaldehyde	–	+	31.5
indole	+	5.0	+
phenylacetaldoxime	+	2.0	+
2-phenylnitroethane	+	0.4	+

oil have been discussed in detail by Buccellato (1981)[3] and Anonis (1985).[4] Buccellato stated: "Many unidentified or discovered but not yet publicized chemicals exist in orange blossom, and in order to gain a proper understanding of orange blossom much additional work is needed."

Anonis wrote: "It is difficult to reproduce the typical odor of the natural neroli oil, although new research has been done on citrus flower oils and a few new aromatics are available. Neroli with its elusive top note and fixative properties, will remain an important note in both women's and men's fragrances."

What is the state of the art of interesting olfactive constituents of orange blossom now? During the last decade excellent studies have been carried out on the headspace, oil and absolute of orange flowers and other resembling flowers.

Already in 1973 Corbier and Teisseire[5] discovered a new constituent in neroli oil namely: 2,5-dimethyl-2-vinyl-4-hexenal. For several years, this compound has been on the market under the tradename of Neroli Aldehyde. According to the producer the chemical reveals a little bit of the topnote of neroli oil. The fruity citrus character is clearly noticeable.

In 1979 Sakurai and coworkers[6] studied the absolute of the flowers of Citrus unshiu, known as the Mikan flower in Japan. They mentioned that the flower of Citrus unshiu is in bloom at the beginning of the summer, and that it has a very delicate floral, fruity, green and somewhat sweet, pungent odor, which wraps powerfully all over the place.

Some new constituents of the absolute are shown in Table I. In 1980 Kaiser and Lamparsky[7] published their detailed results on the constituents of bitter orange flower headspace and the flower absolute. At that time they could only detect 2-methylbutyronitrile in the complex mixture from the flower headspace, from which they collected a very small quantity. They detected several new benzenoid, mono- and sesquiterpenoid nitrogen derivatives in the absolute (see Table I).

Joulain[8] (1986) studied the headspace constituents of the flowers of the false acacia (*Robinia pseudoacacia* L.) and reported that its odor is strongly reminiscent of that of orange blossom. Joulain found the new and some of the same constituents as present in the orange blossom absolutes (see Table I).

Scheme 3

Olfactively Characteristic Compounds in Flowers

Flower Type	Character-Impact Compounds
Rose	Damasc(en)ones and derivatives
Jasmin	Methyl jasmonate and cis-jasmone
Bitter orange	Probably: Nitrogen compounds For example: Schiff bases Methyl anthranilate/neroli aldehyde

More recently Kaiser[9] stated that the orange flower belongs to a group of natural scents with "white floral image." To this group also belong flowers like jasmin, false-acacia, gardenia, and honeysuckle. The group contains mono- and sesquiterpene alcohols and aromatic alcohols and esters as general constituents. Specific constituents for orange flower are indole, methyl anthranilate, 2-phenylnitroethane and phenylacetaldoxime. Finally one may ask, does bitter orange flower contain olfactively characteristic constituents and what are its identities? We believe that bitter orange blossom contains character-impact compounds, but we can only guess their structures (see Scheme 3).

The condensation products of methyl anthranilate with hydroxycitronellal (aurantiol), a so-called Schiff's base, is often used for the reproduction of neroli oil. This product, known for more than 50 years, is completely synthetic and not nature-identical.

The absolute from orange flowers may contain up to 10% methyl anthranilate. Strangely enough, up to now no Schiff's bases with lower aldehydes (say up to C-6) have been found in flower extracts. These condensates have strong long-lasting odors, neroli- and mandarin-like, and could be present in the concrete and absolute.

Bitter Orange Leaf Oils

The most important bitter orange leaf oil is petitgrain bigarade oil.[2] Formerly this oil has been processed from the setting bitter orange fruits, so-called petitgrains or orangettes. The distillate water of the leaves and twigs is called eau des brouts. From the distillate-water an oil can be isolated by extraction, this oil is called orange leaf-water oil or oil of eau des brouts. By extraction of bitter orange leaves with a suitable solvent one may obtain a petitgrain bigarade concrete and, after treatment with ethanol, the absolute.

Isolation and Composition of Leaf Oils—Whereas neroli oil is obtained by hydrodistillation with the flowers in a lot of water, petitgrain bigarade oil is processed from the leaves and small twigs by steamdistillation with a small amount of water. Steam distillation is mostly performed with direct (dry) steam, generated in a separate steam boiler. The yield of the oil can vary between 0.1 and 0.3% depending on the age of the leaves; leaves from young trees in general give higher oil yields.

By extraction of the distillate water from the leaves with a suitable solvent, for instance hexane, one can obtain the oil of eau des brouts in a yield of 0.2% to 0.3%, (seldom higher than 0.5%), which contains the water-soluble part of petitgrain oil.

One can also produce a petitgrain oil by so-called hydrodiffusion.[10] Hydrodiffusion is carried out with low pressure steam (<0.1 bar) replacing the volatiles from the intact plant-material by osmotic action.

A normal steam-distilled petitgrain bigarade oil contains about 25% linalool and ca 50% linalyl acetate; whereas a hydrodiffusion oil consists of 5% linalool and ca 80% linalyl acetate. So it seems that a high proportion of the linalyl acetate present in the leaves hydrolyses and decomposes during steamdistillation. Oil of eau des brouts, isolated from the distillate-water, consisted of more than 95% of oxygen-

Table II. Chemical composition of essential oils from bitter orange tree

Constituent	Neroli oil	Petitgrain bigarade oil	Eau de brout oil	Fruit peel oil	Constituent	Neroli oil	Petitgrain bigarade oil	Eau de brout oil	Fruit peel oil
α-thujene	0.05%	0.02%	-	0.001%	linalool	8.93	20.20	39.12	0.220
α-pinene	0.75	0.19	0.01%	0.383	terpinen-4-ol	0.42	0.15	0.68	0.004
camphene	0.05	0.01	-	0.002	p-cymen-8-ol	-	-	0.05	-
sabinene	2.26	0.40	-	0.162	α-terpineol	3.30	4.00	42.54	0.870
β-pinene	0.52	2.53	-	0.440	nerol	0.82	1.00	1.14	0.009
myrcene	2.49	2.60	0.05	1.733	geraniol	2.18	3.00	3.99	0.016
α-phellandrene	0.04	0.01	-	0.028	1,8(9)-menthadienol-10	-	-	0.11	0.010
δ-3-carene	0.05	0.03	-	0.010					
α-terpinene	0.18	0.06	-	0.020	octyl acetate	-	-	-	0.030
p-cymene	0.22	0.05	-	0.010	decyl acetate	-	-	-	0.004
limonene	12.88	5.43	0.08	93.770					
cis-ocimene	0.82	0.84	0.02	0.050	linalyl acetate	6.37	45.85	0.65	0.426
trans-ocimene	5.60	2.44	0.04	0.270	α-terpinyl acetate	0.06	0.10	0.02	0.025
γ-terpinene	0.33	0.06	0.01	0.005	citronellyl acetate	0.09	0.07	-	0.048
terpinolene	0.42	0.29	0.05	0.006	neryl acetate	1.36	2.15	0.20	0.028
					geranyl acetate	2.65	3.92	0.42	0.142
					1,8(9)menthadienyl acet.	0.02	0.01	-	0.011
2,2,6-trimethyl-6-vinyl -tetra hydropyran	0.04	0.01	0.03	-					
linalooloxide A (furanoid)	0.07	0.06	2.99	-	β-elemene	0.05	0.03	0.01	0.030
linalooloxide B (furanoid)	0.02	0.04	1.61	-	β-caryophyllene	0.54	1.77	0.03	0.103
perillene	0.02	0.01	0.02	-	cis-β-farnesene	0.08	0.08	-	0.033
1,8-cineole	-	-	0.14	-	α-humulene	0.18	0.18	0.04	0.012
limonene oxide I	0.03	-	0.03	0.01	germacrene D	0.05	0.04	-	0.135
limonene oxide II	0.03	-	0.01	0.01	trans-β-farnesene	0.13	0.46	-	0.011
					valencene	0.05	0.03	-	0.005
					δ-cadinene	0.03	0.07	-	0.003
					caryophyllene oxide	0.01	0.04	-	-
octanal	0.02	0.01	0.02	0.065					
nonanal	0.01	-	-	0.017	nerolidol	2.58	0.12	0.02	0.112
decanal	0.03	0.02	0.02	0.130	δ-cadinol	0.02	0.01	-	-
undecanal	-	-	-	0.008	(Z,E)-farnesol	0.04	-	-	0.005
dodecanal	-	-	-	0.020	(E,E)-farnesol	1.48	-	-	0.010
					β-sinensal	-	-	-	0.010
citronellal	0.01	0.05	0.01	0.004					
neral	0.03	0.03	0.01	0.042	nootkatone	-	0.03	-	0.043
geranial	0.10	0.07	0.02	0.036					
perillaldehyde	-	0.01	-	0.020	2-phenylethanol	0.20	0.20	-	-
					indole	0.16	-	-	-
					methyl anthranilate	0.10	0.10	-	-
octanol	-	-	-	0.019	N-Me methyl anthranilate	0.10	0.05	-	-
decanol	-	-	-	0.008	cis-jasmone	0.05	-	-	-
					methyl jasmonate	0.01	-	-	-
α-fenchol	-	-	0.22	-	geranyl acetone	0.05	-	-	-
cis-β-terpineol	-	-	0.18	-					
trans-β-terpineol	-	-	0.21	-	osthol	-	-	-	0.110
borneol	-	-	0.10	-	meranzin A	-	-	-	0.131
γ-terpineol	-	-	1.84	-	meranzin B	-	-	-	0.186

containing monoterpenes, mainly the alcohols linalool (40%), α-terpineol (40%) and linalool oxides (5%).

Olfactive Properties of Leaf Oils and New Constituents—Petitgrain oil is well-known for its fresh, floral, cologne- and bergamot-like odor. The oil is generally known and used for its freshness, probably mainly due to its high content of linalyl acetate (and alcohol). The fine, floral freshness of petitgrain oil, however, is more delicate than that of linalyl acetate alone. The natural freshness of petitgrain oil is more reminiscent of myrcenyl- and ocimenyl acetate than that of linalyl acetate. Surprisingly enough none of these products have yet been found in citrus oils, though myrcenol and ocimenol have been found in essential oils.

Petitgrain oil possesses a natural green odor-aspect, which generally has been put down to traces (ca 25 ppm) of 2-methoxy-3-isobutylpyrazine.

The green odor-facet of petitgrain oil, however, is due to a mixture of alkyl-substituted methoxypyrazines (3-isopropyl-, 3-sec-butyl- and 3-isobutyl-) in a total concentration of 25-50 ppm. Petitgrain oil shows a good performance in extrait perfumes, toilet waters, air fresheners, and even in soap compounds. Its behavior in heavy duty detergent compounds, however, is rather moderate because of its high content of linalyl acetate, which is rather instable in that environment.

Bitter Orange Peel Oil

Isolation and Chemical Composition—Bitter orange peel oil is obtained by cold-pressing or needle puncturing of whole fruits and centrifuging of the oil in yields of 0.4-0.5%.

We studied the chemical composition of peel oil in detail.[11,12] The volatile part of bitter orange oil consists of more than 95% of monoterpene hydrocarbons, with limonene being more dominant (more than 90%).

We investigated the composition of the oil:

- in fully developed unripe fruits (on the tree and picked)
- in ripe fruits (on the tree & picked)
- from processed (low temperature) fruits
- from steamdistilled fruits (whole, peel and pulp)
- from conserved fruits (peel and pulp)

We found that, during ripening:

- lower aliphatics (C-1 to C-6) and sesquiterpene oxygen-derivatives are formed;
- a relation exists between the concentrations of monoterpene hydrocarbon and oxygen-derivatives;

during processing:

- monoterpene hydrocarbon content decreases;
- linalool (and acetate) contents decrease,
- α-terpineol and linalool-oxides are formed.

Olfactive Properties of Peel Oil—The olfactive properties of bitter orange peel oil are strongly citrusy; they are, however, rather different from those of sweet orange oil.

The oil is less aldehydic but more freshly floral, with bergamot-connotations.

Olfactively most important groups of compounds in bitter orange oil are the aliphatic aldehydes, and the oxygen-containing mono- and sesquiterpenes. Especially unsaturated straight chain aldehydes C-8 to C-14, linalool (and acetate), nootkatone and α-selinenone may be mentioned for their odor character (Table II). Bitter orange peel oil is a useful ingredient for flavorists because of its tenacity. The oil is a valuable adjunct in the flavoring of alcoholic and non-alcoholic beverages, confectionary, and baked goods.

Because of the increase in citrus style scents, the peel oil has gained popularity in the fragrance field, for instance, in men's line products such as aftershave and body lotions. It serves well in perfumes, toilet waters and cosmetics, to which it imparts interesting notes.

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