

Bulgarian Zdravetz Oil

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Some of the components of an essential oil have opened the way to the discovery of new groups of compounds with unusual chemical behavior. Our subject, *zdravetz* (*Geranium macrorrhizum L.*), belongs to the Geraniaceae family, which abounds in aromatic plants including the genus *Pelargonium*. It originates from South Africa and is widespread along the Mediterranean coast. Large quantities of *pelargonium* oil, incorrectly known as "geranium oil," are produced from different varieties of *Pelargonium roseum L.* Of the genus *Geranium*, only *zdravetz* is used in Bulgaria for production of essential oil and aromatic extracts.

In Bulgarian "zdrave" means "health." The people gave the name to this mountain plant for its green leaves with their specific warm-fresh aroma. It is a herbaceous perennial plant which grows in Bulgaria mostly at altitudes of 800-1700 metres above sea level. It forms long stems in the soil, branching out in vegetative tips with bunches of green leaves that contain less than 0.1% oil and are used for the preparation of aromatic products. Attempts at industrial cultivation of *zdravetz* have always been unsuccessful.

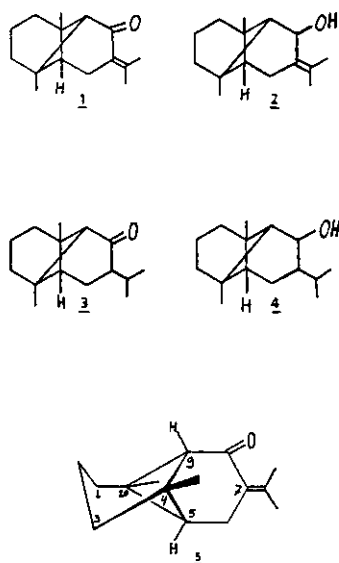
The first small quantities of *zdravetz* oil were produced in Bulgaria around 1926 by water distillation of the fresh leaves. Since 1950, the oil is a regular item in the list of natural Bulgarian aromatics as, in the following years, were the aromatic extracts, i.e. *zdravetz* concrete and *zdravetz* absolute. The total annual amount, produced from wild-growing plants only, is limited to some several

hundred kilograms.

Water distillation of the fresh leaves yields approximately 90% of the oil, while the remaining 10% is separated by cohobation of the distilled water. The end product is a mixture of the two oils in their natural proportions. At ambient temperature, about 70% of the oil volume is taken up by large colourless prismatic crystals with no odour. The blue-greenish semi-viscous liquid portion contains all odourous components causing the unique aroma.

Some thirty years ago, when we started our work on the *zdravetz* oil, almost nothing was known of its chemical composition. The crystal part was wrongly assumed to be a sesquiterpene oxide,^{1,2} and on this basis a completely incorrect structure was proposed in 1952.³ Beside the monoterpene hydrocarbon *terpinolene*, no other individual components were identified in the liquid part of the oil.⁴ The reason was that the oil abounds in sesquiterpenoids which present great difficulties in the investigation of its composition by classical methods for isolation, purification, identification and structure elucidation.

In the early fifties, the chromatographic methods, i.e., paper and thin-layer chromatography followed by gas-liquid chromatography (GLC), were developed and made available to a wide number of users. At the same time, the modern physical methods such as infrared spectroscopy (IR), nuclear-magnetic resonance (NMR) and mass-spectrometry (MS) proved their many advantages in the



identification and structure elucidation of complex and unstable molecules. All this created promising background for a successful investigation of the chemical composition of zdravetz oil.

From the perfumer's point of view, the liquid part of the oil, the "eleoptene," deserves first interest. It contains approximately 2% monoterpenes and 25% sesquiterpenes which were isolated as a mixture by flash-chromatography on silica and were further separated by preparative chromatography on silica impregnated with silver nitrate. The monoterpenes α -pinene, Δ^3 -carene, α -phellandrene, terpinolene, dipentene, γ -terpinene and p-cymene were identified by means of GLC and IR spectra.⁴ The sesquiterpenes α - and β -elemene, caryophyllene, humulene, γ -muurolene, selina-3,7(11)-diene, selina-4(15),7(11)-diene, ar-curcumene, Δ -cadinene, calamenene, α -santalene were identified as individually isolated compounds by means of their IR, NMR and MS data.⁶ About 40% of the eleoptene is a mixture of sesquiterpene alcohols, consisting of juniper camphor, β -eudesmol, junenol and elemol, as well as traces of borneol.⁷

Germazone, A New Sesquiterpene Ketone

By precise chromatographic separation, a new sesquiterpene ketone, germazone, was isolated as a minor component of eleoptene. Germazone is the first representative of a new group, i.e., the germazane group of tricyclic sesquiterpenes. It has a basic *cis*-decaline skeleton with an additional C-4/C-9 junction forming an internal cyclobutane ring. The structure 1 and the stereochemistry of germazone were elucidated on the basis of its spectral data and of those of the derivatives 2, 3 and 4.⁸

The Dreiding model of germazone presents a

rigid molecule, 5. It has a plane of symmetry intersecting the carbonyl and isopropylidene groups and the C-2,5,6 and 9 atoms. The C-5 and C-9 carbon atoms of the cyclobutane ring are pseudo-asymmetric, while the other two, C-4 and C-10, are asymmetric and possess an opposite (C-4R and C-10S) configuration. All this considered, germazone exists as a single optically inactive meso form only.

Germazone is a very suitable model for investigating the stability and acid-catalyzed transformation of cyclobutane systems adjacent to an oxygen function. Thus, instead of the expected saturated alcohol 4, we obtained the bicyclic hydrocarbon *cis*-selinane 6 (figure 1), by catalytic hydrogenation of the alcohol germazol, 2 (PtO₂ in AcOH).⁹ Evidently, the hydrogenolysis of 2 is accompanied by a cleavage of the cyclobutane ring, thus converting the germazone skeleton into a *cis*-selinane one. Treatment of 2 with acids under selected conditions resulted in different *cis*-selinane derivatives.⁹

All products, 7-10, are fairly unstable due to the presence of the conjugated diene system. They are all optically inactive racemates and the cyclobutane cleavage proceeds via both diastereomeric carbocations 11 and 12 with equal possibility for their formation.

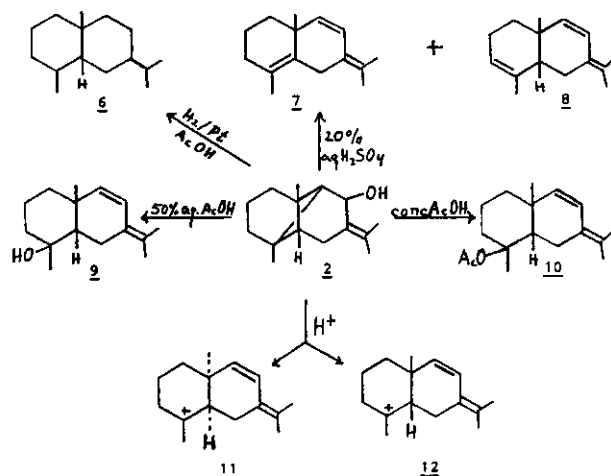


Figure 1

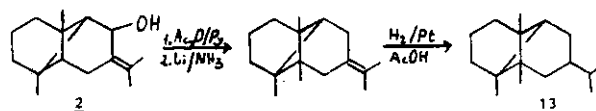


Figure 2

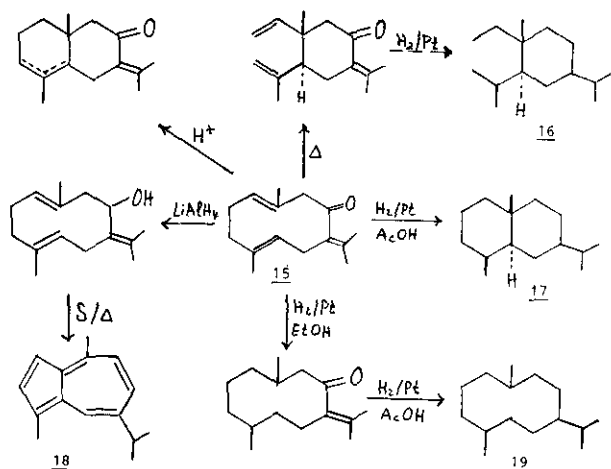
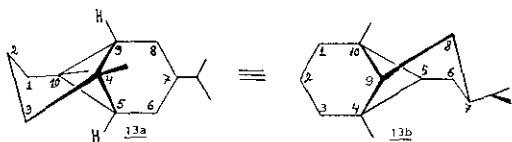


Figure 3

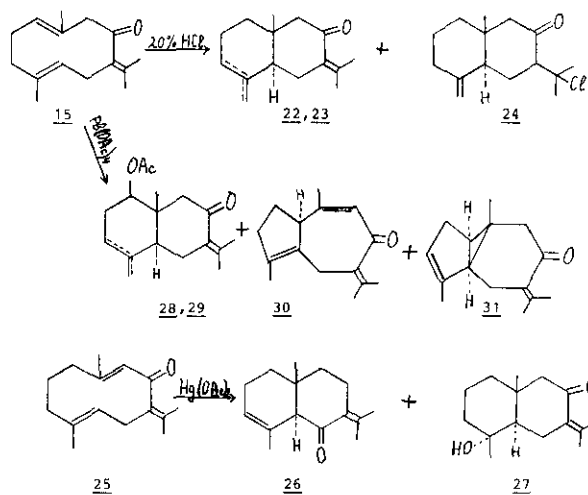


Figure 4

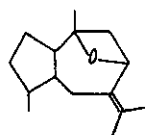
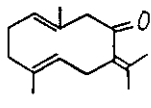
Because of the instability of the cyclobutane ring in acidic medium, the saturated hydrocarbon germazane, 13, was obtained according to figure 2.

Germazane, 13, is optically inactive. Its Dreiding model reveals the high symmetry of the germazane skeleton with two perpendicular planes of symmetry, one intersecting the carbon atoms 2,5,6,8 and 9 (13a), and the other 1,3,4,7 and 10 (13b).

Germacrone

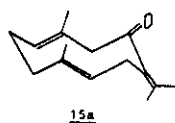
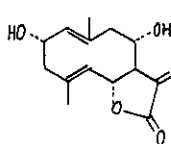
The elucidation of the correct structure **15** of germacrone, the crystalline part of the oil, added new data to our knowledge of the chemistry of sesquiterpenes. Actually, germacrone happened to be the first isolated representative of a new sesquiterpene group—the germacrane group. Later, its key position in the biosynthetic pathways of many mono-, bi- and tricyclic sesquiterpenoids was shown.

Shortly before we commenced our study on germacrone, an incorrect structure, **14**, of a guaiane oxide was published.⁹ The basic flaw in this structure was revealed the very first time we ran the IR spectrum of germacrone. To our surprise and satisfaction, a strong absorption band appeared at 1670 cm^{-1} , typical for an α,β -unsaturated ketone, but impossible for an oxide with a supposed structure **14**. This prompted the immediate change of the former name "germacrol" to the chemically correct "germacrone."

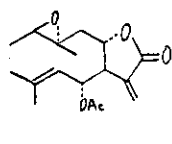
**14**Germacrol⁹**15**Germacrone¹⁰

The correct structure **15** with germacrane carbon skeleton was established by means of chemical transformations and degradation, some of which are shown in figure 3, and by wide use of IR spectra for identification of the products.¹⁰

The sesquiterpene lactone pyretrosin has the same germacrane skeleton. Its structure **21** was proposed by Barton in 1957.¹¹ In view of the knowledge existing at that time (1953-1957), it was certainly confusing to have conversion of one and the same starting compound into the four hydrocarbons **16-19** with different carbon skeletons, three of which were already well-known (**16**, **17** and **18**), while **19** was a new one. Experiments by X-ray crystallography¹² confirmed the structure **15** and showed also that in the crystal state the germacrone molecule

**15a****20**

Tatrudin B

**21**

Pyretrosin

has a crown conformation, **15a**, with crossed *trans* double bonds. This conformation has been accepted for the germacrone analogues as well.

The discovery of the germacrane sesquiterpene carbon skeleton and the typical cyclization reactions established by germacrone gave rise to a tidal wave of investigations in three main directions.

The first was a large-scale and detailed search for the other natural products with germacrane skeleton. The results, produced by a large number of investigators, by far exceeded all expectations. To this moment, more than six hundred individual germacrane derivatives have been isolated from essential oils and plant extracts. Some of them are unsaturated hydrocarbons or oxygenated compounds, related to germacrone. The major part is a large variety of sesquiterpene lactones with germacrane skeleton, for example germacranolides of the types **20** and **21**, found mainly in different plants of the family Compositae. Some of them possess biological activity, as cytotoxic, antimicrobial, cardiovascular, or are considered to be chemotaxonomic markers.¹³

The second direction was intensive investigation of the reactivity of the unsaturated germacradiene system as it was represented by the germacrone molecule. It was shown that the three different ways of cyclization to compounds with elemene, **16**, selinane, **17**, and guaiane, **18**, skeletons are common for all germacra-1,4-dienes and for many of their analogues. This is a result of the specific location of the two endocyclic double bonds, positioned close and cross-wise to each other in the preferred "crown" conformation, **15a**.

The type of the products depends on the agent used for the cyclization. For example, 20% aqueous hydrochloric acid¹⁴ converts germacrone, **15**, to the selinenones **22**, **23** and **24** (figure 4). Mercury-II-acetate converts isogermacrone, **25**, to **26** and **27**.¹⁵

On the other hand, lead-IV-tetraacetate converts germacrone to the products **28**, **29**, **30** and **31**,¹⁶ two of which are a result of the rarely occurring cyclization to guaiane derivatives.

UV irradiation is another method of initiating chemical reactions. In this way, isogermacrone, **25**, was converted¹⁷ to a mixture of germazone, **1**, and tricyclic compounds of the type **32**. This is a good confirmation of our former suggestion that the isogermacrone molecule with the appropriate configuration, **25a**, could be the precursor of germazone (figure 5).

These results provoked some of our colleagues to a new investigation of the composition of the petrol extract from fresh zdravetz leaves, prepared under very mild laboratory conditions. It was shown that the extract contains small quantities of isogermacrone and of the tricyclic ketones, **32**. Hence, all

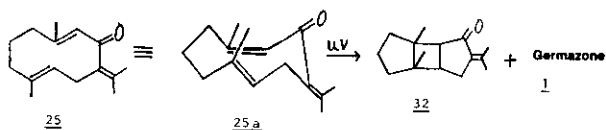


Figure 5

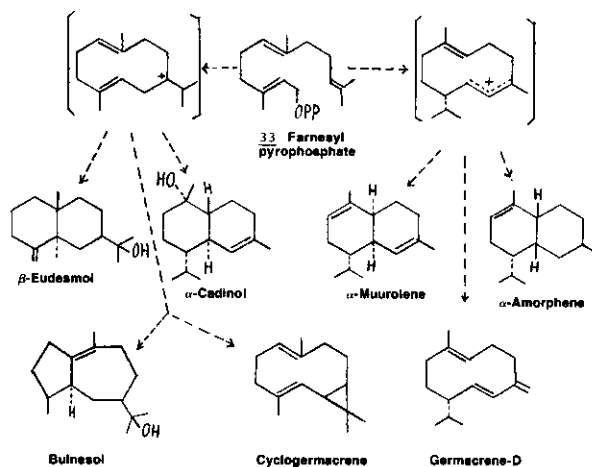


Figure 6

these sesquiterpenes, isogermacrene, germazone and the ketones, 32, are natural components of the leaves. It is still not clear if they are produced from germacrene under solar irradiation, or by its enzymatic transformation in the living leaves.

A third trend of investigations concentrated on clarifying the participation of germacrene-type compounds in the biosynthetic pathways of some cyclic sesquiterpenoids. The already discussed *in-vitro* cyclizations of germacrene support the assumption that unsaturated germacranes play a key role in the *in-vivo* cyclizations.

All biogenetic pathways of terpenoids include unsaturated pyrophosphate intermediates which are capable of extending the carbon chain or taking part in cyclizations. Geranyl-pyrophosphate is the key intermediate of monoterpenes. Extension of the chain with an isoprene unit gives farnesyl-pyrophosphate, 33, which is the active molecule for further conversions to all known sesquiterpenes (see figure 6). Of them, germacranes, eudesmanes, guaianes and cadinanes originate from cyclodecadiene cations with different geometry of the double bonds. Examples are given in figure 6.

Once created, these bicyclic carbon systems, as well as the starting germacradiene one, are subjects of further biogenetic transformations to other sesquiterpenes via the corresponding carbon-cation precursors (figure 7).

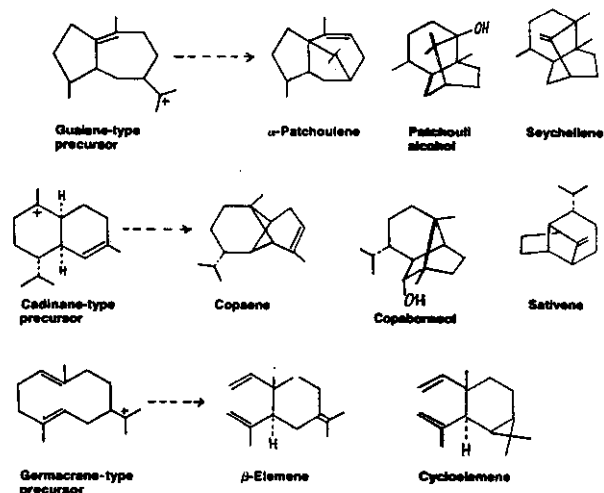
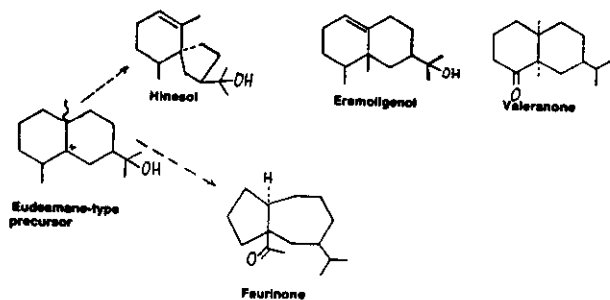


Figure 7

Characteristics of Zdravetz Oil

Zdravetz oil, according to the Bulgarian State Standard, has the following characteristics:

- Appearance—semi-solid mass with white crystals and green-blue color
- Density, 40°C—0.9380-0.9680
- Refractive index, 40°C—1.5000-1.5190
- Optical rotation, 40°C, 100mm tube— -1° to -9°
- Acid number, max.—3
- Ester number—3.5-8.0
- Acetyl number—20.7-50.0
- Melting point, °C—34-50
- Free alcohols (as geraniol), %—4-12
- Combined alcohols (as geraniol), %—1-4
- “Stearoptene” (i.e., germacrene), %—42-53

These characteristics, i.e., too high density and refractive index, both determined at 40°, point to an oil rich in oxygenated compounds. We already know that the oil contains approximately 90% sesquiterpenoids, of them about 50% are germacrene.

The latter is traditionally shown as “stearoptene,” and the same stands for the alcohols calculated as geraniol in spite of its absence in the oil. The low acetyl value, as compared with the relatively higher alcohol content established by detailed chemical analysis, confirms their tertiary nature beyond doubt.

According to perfumers, the specific aroma of the oil blends perfectly with amber, lavender and oak moss to create perfumes with sunny warm notes, mountain freshness and, depending on the composition, with veiled or more distinct and appealing oriental shades. The fixative properties of the oil gained the perfumes universal acceptance.

Summary

I believe that this review has, first of all, supplied all current information on the Bulgarian zdravetz oil used as an essential oil in perfumery. I hope also to emphasize how very important detailed chemical investigations are in providing new basic scientific results and in clearing the way to the better understanding of the immense creative forces of nature.

Acknowledgement

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