# Comparative Investigation of Essential Clover Flower Oils from Austria Using Gas Chromatography−Flame Ionization Detection, Gas Chromatography−Mass Spectrometry, and Gas Chromatography−Olfactometry

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The essential oils of red and white Austrian clover flowers (*Trifolium pratense* L. and *Trifolium repens* L.) were analyzed by GC−FID, GC−MS, and GC−olfactometry (GC−sniffing technique). More than 50 compounds were identified in the samples. The main constituents (concentration higher than 2%) of the investigated samples were maltol (8.2%), linalool (4.2%), 1-phenylethyl alcohol (3.2%), phenol (2.9%), phenylethyl acetate (2.7%), acetophenone (2.4%), and (*Z*)-3-hexenyl acetate (2.2%) for red clover flowers and maltol (5.3%), linalool (3.8%), phenol (3.6%), phenylethyl acetate (3.3%), and 2-phenylethyl alcohol (2.8%) for white clover flowers. The odor of these samples has been characterized, and a correlation compound/odor impression is given.

**Keywords:** *Clover flowers (red and white); essential oil; GC−FID; GC−MS; GC−sniffing technique; odor; fragrance; green fodder*

## INTRODUCTION

The volatiles of red and white clover flowers were analyzed by some authors, in past years (Jakobsen and Olsen, 1994; Kameoka et al., 1977; Kami, 1978), especially to find out those constituents responsible for pheromone effects (Buttery et al., 1984; Kamm and Buttery, 1986). A comparative investigation of odorous compounds of *Trifolium pratense* and *Trifolium repens* from Austria (cultivars) using GC−FID, GC−MS, and GC−olfactometry has not been carried out yet. Therefore, it was our aim to analyze the volatiles and identify those compounds responsible for the complex odor impression of these clover samples.

## EXPERIMENTAL PROCEDURES

**Materials.** Red and white clover flowers (*T. pratense* L. and *T. repens* L.) from cultivars were collected in lower Austria in August 1995. The essential oils were obtained by exhaustive steam distillation (5 h) (yield of red clover oil, 0.17%; yield of white clover oil, 0.11%).

**Organoleptic Testings.** Ten microliters of each oil sample was placed on an odor strip, and the odor was characterized by perfumers.

**GC−Sniffing Technique.** A Fractovap 2101 GC instrument with split system and an LT Programmer 230, an Electrometer 160 instrument (Carlo Erba Co.), and a Kompensograph III recorder (Siemens Co.) were used. The column was a 25 m low-polarity FSOT-RSL-200 fused silica gel column (0.32 mm i.d., 0.2 $\mu$m film thickness; Bio-Rad Co.). Conditions were as follows: detector temperature (FID), 320 °C; injector temperature, 250 °C; sniffing capillary heating, 250 °C; temperature program, 40 °C/5 min at a rate of 10 °C/min to 230 °C/20 min; $H_2$ carrier gas, 2 mL/min; splitless mode, sniffing split ratio, 1:50 (FID detector:nose). The peak/odor impression correlations were performed by four fragrance chemists.

**Gas Chromatography.** The volatiles were separated in a GC 14A instrument with a C-R6A integrator (both Shimadzu Co.). For other parameters, see GC−Sniffing Technique.

**Gas Chromatography−Mass Spectrometry.** A GC 17A instrument in connection with a QP-5000 mass spectrometer (both Shimadzu Co.) was used. This system was under control of the data system ProLinea 4/50 (Compaq Co.). Operation parameters for mass spectra registration were as follows: mass range, 35−450 amu (EI mode, 70 eV; acquisition cyclus time, 0.35 s); carrier gas, helium; interface heating, 220 °C. For other parameters, see GC−Sniffing Technique. MS spectra of the detected compounds were identified by correlation with NBS and WILEY (on-line) or FOOD and NIST (off-line) MS library. Many nonterpenic constituents (labeled in Table 1) could be identified also by GC−FID by coinjection of pure substances and correlation of their Kovats indices according to the method of Jennings and Shibamoto (1980).

## RESULTS AND DISCUSSION

More than 70 compounds in the essential oils of red and white clover flowers were detected by GC−FID, and the structures of 53 constituents (including less odor relevant fatty acids and their esters as well as higher hydrocarbons) were identified by GC−MS (Table 1). The main components (higher than 2%, excluding fatty acids and their esters or higher hydrocarbons) are as follows: in the red clover essential flower oil, maltol (8.2%), linalool (4.2%), 1-phenylethyl alcohol (3.2%), phenol (2.9%), phenylethyl acetate (2.7%), acetophenone (2.4%), and (*Z*)-3-hexenyl acetate (2.2%); and in the white clover essential flower oil, maltol (5.3%), linalool (3.8%), phenol (3.6%), phenylethyl acetate (3.3%), and 2-phenylethyl alcohol (2.8%).

The chemical compositions of both samples are very similar, but there are detectable changes in the concentrations of the aforementioned main compounds. This result, as well as the appearance of odorous constituents not found in clover samples until now, explains the peculiar, but for the perfumer's nose, interesting odor impression of Austrian essential *Trifolium* oils. Contrary to published data (Kami, 1978), lovage-like odor is dominating the red and white essential oils, and the odor impression is described as follows: red clover essential oil, lovage-like, spicy, iris absolute-like, vetiver-like without pyrazin notes, week

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**Table 1. Volatiles in the Essential Oil of Red Clover Flowers (EORCF) and White Clover Flowers (EOWCF) from Austria**

| compound[a] | % peak area GC−FID EORCF | % peak area GC−FID EOWCF | Kovats index[b] | odor |
|---|---|---|---|---|
| (*Z*)-1-hexen-3-ol | 1.3 | 1.7 | 769 | green, grassy |
| (*E*)-2-hexenal | 1.2 | 0.2 | 839 | green-fruity |
| (*Z*)-3-hexenol | 0.8 | 1.1 | 842 | green-fatty |
| (*E*)-2-hexenol | 0.4 | 0.9 | 849 | green-fruity |
| hexanol | 1.6 | 1.2 | 855 | winey-fruity |
| benzaldehyde | 0.7 | 1.2 | 944 | almond |
| 2-ethylhexanal | 0.2 | 0.9 | 957 | mild-fruity |
| 1-octen-3-ol | 1.7 | 0.9 | 962 | mushroom |
| (*Z*)-3-hexenyl acetate | 2.2 | 1.3 | 989 | green-fruity |
| phenol | 2.9 | 3.6 | 1007 | phenolic |
| ocimene | 0.7 | tr[c] | 1022 | warm-herbaceous |
| limonene | 1.6 | 0.9 | 1027 | fresh-citrus |
| benzyl alcohol | 0.9 | 1.8 | 1031 | faint-floral |
| acetophenone | 2.4 | 1.6 | 1044 | pungent-sweet |
| (*Z*)-linalool oxide | 0.9 | 0.7 | 1070 | floral-lavender |
| (*E*)-linalool oxide | 1.1 | 1.3 | 1082 | floral-lavender |
| linalool | 4.2 | 3.8 | 1091 | floral-lavender |
| 1-phenylethyl alcohol | 3.2 | 1.9 | 1099 | warm-floral |
| 2-phenylethyl alcohol | 1.9 | 2.8 | 1104 | floral-rose |
| maltol | 8.2 | 5.3 | 1108 | sweet-fruity |
| benzyl acetate | 1.3 | 0.8 | 1142 | sweet-floral |
| phenylethyl acetate | 2.7 | 3.3 | 1230 | floral |
| linalyl acetate | 0.8 | 1.4 | 1238 | floral-lavender |
| geraniol | 0.2 | 1.3 | 1244 | floral-sweet |
| cinnamic aldehyde | 1.1 | 0.4 | 1252 | cinnamon-spicy |
| cinnamic alcohol | 1.3 | 0.9 | 1298 | warm-balsamic |
| methyl cinnamate | 0.9 | 1.2 | 1361 | fruity-balsamic |
| coumarin | 1.3 | 1.4 | 1414 | fresh-hay |
| $\beta$-caryophyllene | 0.3 | 1.1 | 1421 | woody-spicy |
| 3-butylphthalide | 1.2 | 1.6 | 1423 | warm-spicy |
| ethyl cinnamate | 1.5 | 0.2 | 1441 | sweet-balsamic |
| cinnamyl hexanoate | 0.8 | 1.1 | 1809 | floral-balsamic |
| fatty acids and their esters (12 compounds) | 27.8 | 25.3 | | |
| higher hydrocarbons (higher than $C_{16}$, 9 compounds) | 19.7 | 25.7 | | |
| unidentified compounds | ~1 | ~1 | | |

[a] In order of their elution (GC). [b] 25 m RSL-200 column. [c] Trace compound less than 0.1%.

green and floral, woody (direction of patchouli), phenolic, and burned side notes; and white clover essential oil, lovage-like, more clean, floral and clear top note, animalic-earthy side notes (direction of ambra), warm, breadlike, and phenolic.

Using a GC−sniffing technique, a significant correlation compound/odor effect was possible: maltol and 3-butylphthalide (genuine component of lovage and celery seeds) are responsible for the lovage-like odor impressions; $C_6$ compounds, coumarin, and some monoterpenes (like limonene, linalool, and linalyl acetate) as well as aromatic constituents for the floral, clean, green, and clear odor impression; cinnamic derivatives for the spicy impressions; and the other compounds for the woody (e.g., $\beta$-caryophyllene), animalic-earthy (e.g., 1-octen-3-ol), warm (e.g., acetophenone), and burned (e.g., higher hydrocarbons) characteristics.

In summary the presented investigation on volatiles of essential oils of *T. pratense* and *T. repens* from Austria led to a surprising result: The essential oils from Austrian clover flowers possess a various composition (e.g., maltol, linalol butylphthalide, and coumarin were found as main constituents in clover samples for the first time) in correlation to published data. Although known odor compounds of clover oils like acetophenone, cinnamates, alcohols, and caryophyllene (Buttery et al., 1984; Kameoka et al., 1977; Kami, 1978; Kamm and Buttery, 1986) were also identified, a significantly different odor impression of the essential oils of Austrian clover flowers was perceived. We found that the essential oil of Austrian clover flowers contain several interesting components which impart an odor impression different from other clover samples.

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