Investigations on Less Volatile Hay Blossom Constituents

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Hay Blossoms (Austrian), GC-MS, GC-FTIR, LC-MS

Five samples (headspace, steam distillation, Likens-Nickerson, methanolic and dichloromethane extracts) of Austrian hay blossoms have been investigated by chromatographic-spectroscopic methods and led to the identification of several non or less volatile compounds (mainly in the Likens-Nickerson extract).

Introduction

Hay blossoms (Flores graminis, Graminis flos) are very useful in phyto- and aromatherapy [1–6]. Because of their heterogeneous composition (hay blossoms consist of the waste-product of hay storage which settles on the ground – it comprises of blossoms, fruit and those parts of meadow grasses and plants above ground) the investigation of their constituents is not an easy task – however mostly the volatile constituents have been investigated [7] and require a great deal of analytical techniques [8].

Results and Discussion

The use of coupling systems like GC-MS, GC-FTIR and LC-MS allows the identification of many components without chemical reactions of the complex mixture of different plants of hay blossoms (botanical composition see Table I).

Table I. Plants of hay blossoms (Austrian).

A) Grass plants: *ca.* 70%, mainly:

Lolium perenne L., Poaceae; *Bromus hordeaceus L. (Bromus mollis L.)*, Poaceae; *Festuca pratense Huds.*, Poaceae; *Poa pratense L.*, Poaceae; *Anthoxanthum odoratum L.*, Poaceae; *Elymus repens (L.)* Gould, syn. *Agropyron repens (L.)* Beauv., Poaceae

B) Other plants: *ca.* 30%, mainly:

Thymus serpyllum L. (Thymus pulegoides L.), Lamiaceae; *Achillea millefolium L.*, Asteraceae; *Taraxacium officinale Web.*, Asteraceae; *Equisetum arvense L.*, Equisetaceae; *Trifolium pratense L.* and *Trifolium repens L.*, Papilionaceae

The investigation of the volatile constituents, which are of high interest and importance for the aromatherapy, led to the identification of mainly terpenic compounds (mono- and sesquiterpenes) [7]. To gain more insight into the chemical composition beyond these mono- and sesquiterpenic constituents (essential oil content of the different samples: headspace: 95%, steam distillation: 67%, Likens-Nickerson: 23%, methanolic extract: 11% and dichloromethane extract: 14%), it seemed to be worthwhile to registrate and analyze also the less volatile compounds.

In five samples (methanol, dichloromethane, Likens-Nickerson (pentane) extracts, steam distillation and headspace sample) it was possible to detect nearly 50 less volatile compounds with a molecular weight higher than 220 and having more than 15 C-atoms. 27 of these hay blossom constituents are shown in Table II. The correlation of data from investigations by capillary gas chromatography (retention times) and by coupled systems, GC-MS, GC-FTIR and LC-MS, resulted in the identification of alkanes, fatty acids and steroic compounds (displayed in Table II with information about the occurrence and concentration of each compound in the different samples). In the case of further more than 20 components it was not clearly possible to provide their structure (most of these hay blossom constituents are alkenes or alkanes and possess a branched carbon side-chain). Also some well-known coumarins (imperatorine: main sedative compound of hay blossom bath, umbelliferone, aesculetine and aesculine) could be found in the Likens-Nickerson, methanolic and dichloromethane extract [2, 7].

In conclusion it proved to be necessary to use various coupled systems (GC-MS, GC-FTIR and LC-MS) in combination with capillary gas chro-

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Table II. Components of hay blossoms with more than 15 C-atoms.

Component	Formula	mwt	sample 1	2	3	4	5
2,2,4,4,6,8,8-Heptamethylnonane	$C_{16}H_{34}$	226	0	0	l	l	l
n-Octadecane	$C_{18}H_{38}$	254	0	l	l	l	l
Palmitoic acid	$C_{16}H_{32}O_2$	256	l	m	h	h	m
n-Octadecanol	$C_{18}H_{38}O$	270	0	l	m	l	l
Linoic acid	$C_{18}H_{32}O_2$	280	l	l	m	m	m
Eicosane	$C_{20}H_{42}$	282	0	0	l	m	m
Oleic acid	$C_{18}H_{34}O_2$	282	0	l	l	m	m
Nonadecanol	$C_{19}H_{40}O$	284	0	l	l	l	l
Stearoic acid	$C_{18}H_{36}O_2$	284	l	l	m	m	m
Heneicosane	$C_{21}H_{44}$	296	0	0	l	m	l
n-Docosane	$C_{22}H_{46}$	310	0	0	l	l	0
n-Octadecyl acetate	$C_{20}H_{40}O_2$	312	0	0	l	l	0
n-Pentacosane	$C_{25}H_{52}$	352	0	0	l	0	0
n-Hexacosane	$C_{26}H_{54}$	366	0	0	l	l	l
Cholestadiene	$C_{27}H_{44}$	368	0	0	l	l	0
n-Heptacosane	$C_{27}H_{56}$	380	0	0	l	0	l
n-Octacosane	$C_{28}H_{58}$	394	0	0	l	l	l
n-Nonacosane	$C_{29}H_{60}$	408	0	0	l	0	0
Squalene	$C_{30}H_{50}$	410	0	0	l	l	l
Gorgostane	$C_{30}H_{52}$	412	0	0	l	0	l
Stigmasterol	$C_{29}H_{48}O$	412	0	0	l	l	l
β-Sitosterin	$C_{29}H_{50}O$	414	0	0	l	l	l
β-Tocopherol	$C_{29}H_{48}O_2$	416	0	0	l	l	l
Lupenol	$C_{30}H_{50}O$	426	0	0	l	0	0
β-Amyrin	$C_{30}H_{50}O$	426	0	0	l	l	l
Ursenol	$C_{30}H_{50}O$	426	0	0	l	l	0
Lanosterol	$C_{30}H_{50}O$	426	0	l	l	l	l

mwt = molecular weight; h: high concentration (more than 30%); m: medium concentration (10 to 30%); l: low concentration (less than 10%); 0: no detection; 1: headspace sample; 2: steam-distillation sample; 3: Likens-Nickerson (pentane) extract; 4: methanolic extract; 5: dichloromethane extract.

matography to identify most of the complex system of volatile, less volatile and non volatile constituents of hay blossoms.

Experimental

The hay blossoms were obtained from Mag. Kottas-Heldenberg & Sohn Ltd., 1010 Vienna, Austria and the p.A. solvents from Fluka Ltd.

GC: A HRGC-Mega-Series from the Carlo Erba Comp., 25 m HP-5 fused silica capillary column (0.32 mm i.d., 0.17 micrometer film) from Hewlett-Packard Ltd. Temp.-prog.: 60–260 °C, 6 °C/min.; injector: 260 °C; detector (FID): 280 °C.

GC-MS: A Finnigan MAT CH7A-Mass spectrometer with a Varian Aerograph-3700 GC; modified EI-FI-Ion source (ion source heating: 250 °C); interface heating: 280 °C, 70 eV, recorded mass range: 35–450 amu at cyclus time 0.6 sec.; OCI from Carlo Erba; column as above.

GC-FTIR: A HP-5890A-GC with HP-5965A-IRD (MCT-detector); 800–4000 cm^{-1}; column as above.

LC-MS: A HP-5988A-LC-MS-System with a HP Magic Interface; particle beam; scan mode: scan acquisition 45.00 to 450 amu and electron multiplier with 2.0 E+002 volts relative; ion source heating: 250 °C; interface heating: 280 °C. Column: SP-5%-ODS 250X4.

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