CONSTITUENTS OF THE LIVERWORT *Lophocolea heterophylla**

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Dedicated to Professor Holger Erdman on the occasion of his 80th birthday.

From *Lophocolea heterophylla* sesquiterpene hydrocarbons β-barbatene, *ent*-β-selinene, cuparene, calacorene, calamenene and cadalene were obtained; α-barbatene is probably an artifact. *ent*-Isoalantolactone is the main sesquiterpenoid component. The characteristic smell of the plant is due to the presence of a minute quantity of the alcohol $C_{12}H_{20}O$, not characterized in details. Nonacosan-10-one and esterified nonacosan-10-ol are present in the lipid part of extract, in addition to "normal" components (n-alkanes, cerides).

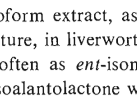
The components of the liverwort *Lophocolea heterophylla* (SCHRAD.) DUM. (*Jungermaniales*) have not been studied intensively as yet. The sesquiterpene hydrocarbons and the composition of other lipidic components in the sporophytes of this liverwort cultivated on an artificial medium, were studied by Thomas[1,2]. The presence of longifolene, caryophyllene, α-selinene and α-cadinene was presumed on the basis of GLC analysis only. Huneck and coworkers[3] published the GLC record without any further interpretation, and other citations[4-6] do not deal with sesquiterpenoids and waxy substances.

As a preliminary essay we have analyzed using GLC, a series of samples of the hydrodistilled essential oil originating from very different habitats. We were able to state their composition not varying qualitatively, and with only small differences in quantity of most components. For preparative studies thoroughly selected plant material was used, and the neutral components were partly steam-distilled, partly extracted from the air-dried material using chloroform. Single compounds were isolated by the combination of column chromatography, TLC and preparation scale GLC. Their identification on the basis of GLC retention times was confirmed using the spectral data (mostly IR spectroscopy and mass spectroscopy) by direct comparison with those of authentic samples and/or literature data.

* Part CCLXXI in the series On Terpenes; Part CCLXX: Parfum. Cosmet, in press.

The main constituents of the sesquiterpene hydrocarbon fraction are β-barbatene (*I*) and *ent*-β-selinene (*III*). The following minor sesquiterpenes, very often occurring in other *Jungermaniales*, were found: α-Barbatene (*II*), cuparene (*IV*), *cis*-calamenene (*V*), calacorene (*VI*) and cadalene (*VII*). Further four minor sesquiterpenes, all with $M^+ = 204$, remained unidentified. α-Barbatene (*II*) seems to be an artifact only, as during the preparative GLC an easy isomerisation of β- into α-derivative was observed. The presence of three derivatives of the cadinane type, differing in successive dehydrogenation, (*i.e.*, calamenene, calacorene and cadalene) is also interesting; the first member of this series – a cadinene derivative – was missing. According to these facts the occurrence of the sesquiterpene hydrocarbons in *L. heterophylla* is rather different in comparison with the data by Thomas[1].

The main component of the oxygenated sesquiterpenoid fraction of the essential oil was *ent*-isoalantolactone (*VIII*), forming the most significant compound of the chloroform extract, as well. This is the first known occurrence of this compound in Nature, in liverworts the existence of sesquiterpene lactones of related structures, very often as *ent*-isomers, is common (26 compounds were registered[7] in 1979). *ent*-Isoalantolactone was accompanied by a very limited amout of an isomer which could not be obtained pure; the fragmentation of this isomer in mass spectrum is very similar to *VIII*. Further minor sesquiterpenoids containing oxygen were a keto-derivative $C_{15}H_{22}O$ and two alcohols $C_{15}H_{24}O$.



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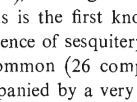

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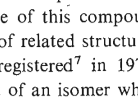

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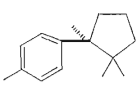

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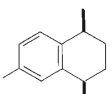

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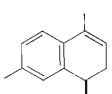

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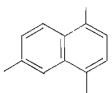

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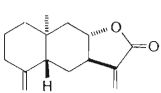

VIII

The striking property of *L. heterophylla* is characteristic and very intensive "mossy" smell manifesting just by any mechanical contact with this plantlet. During the isolation the smell-bearing components formed the low boiling part of oxygen-containing fraction of the essential oil. The sensoric evaluation of a GLC analysis of this essential oil fraction revealed the rich variety of the present fragrant compounds formed by a minimum of 18 components. In contrast to many liverworts in which the bornyl acetate represents the typical fragrant component, the smell of *L. heterophylla* is mostly due to a compound which is present in very limited amounts (~0·003%). We obtained a sample by preparative GLC. According to mass spectroscopy the compound has formula $C_{12}H_{20}O$, and the fragmentation suggests the character of an alcohol.

Although the production of various aliphatic compounds is typical[8] for the plant metabolism, not much information is available on lipids of liverworts[2,9–15]. Similar to the secondary metabolites, the composition of lipids and cuticular components is of some value for chemosystematic evaluations of higher plants and liverworts[16].

After rechromatography on silica gel (impregnated with silver nitrate), the chloroform extract of *L. heterophylla* contained in its hydrocarbon portion the homologous series of *n*-alkanes in the range C_{15}—C_{25}. Similarly to other liverworts[10–14], these alkanes do not show the predominancy of the odd homologues over the even ones, and the highly populated homologues (C_{19}—C_{21}) are lower than common with vascular plants (normally C_{27}—C_{33}). *n*-Alkane GLC record registered is superposed over a "hump"[11,17] – a diffuse peak representing more than 50% of the registered area. Along the n-alkanes the presence of branched alkanes followed from this GLC.

The more polar second fraction yielded a compound not known in liverworts yet, nonacosan-10-one, identified by its mass spectrum. This fraction contained predominantly simple wax esters (cerides) in the range C_{38}—C_{48}; the main component was the ester C_{38}. The transesterification yielded methyl esters of fatty acids C_{12}—C_{26} and alcohols C_{18}—C_{26}. Palmitic and steric acids and docosanol were predominant. No unsaturated compounds were found. From the facts mentioned and from the evaluation of the mass spectroscopy data it follows that the main ceride in the wax is docosanyl hexadecanoate.

The third fraction contained more complex and more polar esters. After transesterification, a systematic GLC and mass spectroscopy analysis proved the presence of primary aliphatic alcohols (C_{13}—C_{26}), and as the main component (over two thirds of the whole fraction) the secondary nonacosan-10-ol. This alcohol, known from the liverwort *Bazzania pompeana*[18], is a congener of the above mentioned nonacosan-10-one. Not yet fully defined hydroxyacids are along with the normal fatty acids (C_{12}—C_{24}) the acidic components of this wax.

The data obtained for *L. heterophylla* show a quite common picture of liverworts

which are members of the order *Jungermaniales*: Considerable number of sesqiterpenic hydrocarbons and relatively high content (~0·3% of dry weight) of *ent*-isoalantolactone which should be of ecologic importance. It is known that the presence of sesquiterpenic lactones (*e.g.* of alantolactone)[19] protects the plant – as antifeeding agents – against herbivorous animals. The "cuticular" components are of some interest but in absence of a true cuticula their localisation and function in the plant protection remains unspecified. For practical purposes of perfumery, the nature of the very intensively fragrant component could be of interest.

EXPERIMENTAL

Plant Material

The specimens of *L. heterophylla* were collected in four different regions of Bohemia (Middle Bohemia, Šumava Mountains, Bohemian Forest and Isera Mountains in Northern Bohemia), all of them during the spring or autumn vegetation period.

Essential Oil

a) The samples for comparative GLC analysis were obtained from thoroughly purified specimens of the fresh plant (cca 30–50 g) using hydrodistillation on Clevenger apparatus[20], time of distillation was 2 h. Essential oils obtained were extracted into n-pentane, dried with sodium sulphate and characterized by their GLC record (Fig. 1).

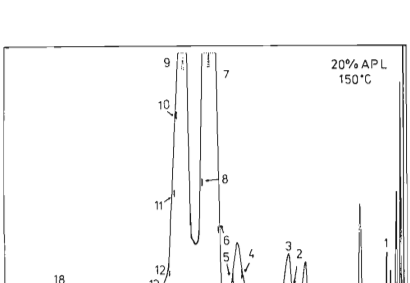

FIG. 1

GLC record of the essential oil from *L. heterophylla*: 1 Bornyl acetate, 4 "fragrant alcohol", $C_{12}H_{20}O$, 5 α-barbatene, 7 β-barbatene, 9 *ent*-β-selinene, 10 cuparene, 11 *cis*-calamenene, 12 ketone $C_{15}H_{24}O$, 13 α-calacorene, 14 cadalene, 15 alcohol $C_{15}H_{26}O$, 16 alcohol $C_{15}H_{24}O$, 17 dibutyl phthalate (!), 18 *ent*-isoalantolactone, 19 isomer of *VIII*; 2, 5, 6, 8 sesquiterpenes $C_{15}H_{24}$, 3 sesquiterpene $C_{15}H_{22}$

b) For preparation of pure compounds, the essential oil was obtained from plant material cleaned by hand sorting and washed of soil. In a typical run, 363 g of fresh plant (corresponding to ~90 g of dried material) were distilled using overheated steam (110°C) and the yield was 0·295 g (0·08% of the fresh plant weight) of nicely smeeling, light green essential oil. A crude polarity separation effected by column chromatography (silica gel) yielded 150 mg of less polar mainly sesquiterprene hydrocarbons, and 120 mg of oxygen-containing fraction ("fragrant fraction"); the latter contained a very limited amout of sesquiterpene lactones.

Sesquiterpene hydrocarbons of the essential oil were isolated using preparative GLC, and were identified by comparison of their retention data, IR and mass spectra with those of standard specimens; minor components were identified only using the GC-MS analysis.

The fragrant fraction was analyzed (repeatedly in several runs) by means of GLC (18 small--bearing peaks) with a very limited amount of bornyl acetate (peak No 1). The characteristically smelling peak No 4, obtained as an oil which polymerised in a short time, and was characterized by its mass spectroscopy (M^+ 180, $C_{12}H_{20}O$, *m/z* 81, 95, 109, 111 [base peak], 147 [M−15−18], 151 [M−29], 162 [M−18], 165 [M−15]).

Chloroform Extract

Air dried liverwort (190 g) was percolated three times with chloroform (1 000 ml in total), and the extract (~3·4 g) was separated by column chromatography on silica gel (100 g) into fractions containing hydrocarbons (fraction 1; 190 mg), wax esters (fraction 2; 120 mg), complex wax esters (fraction 3; 65 mg), and crude *ent*-isoalantolactone (*VIII*) (fraction 4; 590 mg). By column argentation chromatography (hexane) the fraction 1 yielded the mixture of aliphatic hydrocarbons (80 mg), and prevalently the set of sesquiterpene hydrocarbons (95 mg) as above. The homologous series of n-alkanes was analysed by GLC as given before[11,12]; in this case the record exhibited the unimodal type of n-alkanes distribution pattern[21] (C_{15}—C_{25} without the predominance of odd members). Fractions 2 and 3 were transesterified with methanol and gaseous hydrogen chloride in sealed ampules in tetrachloromethane[11,21]. Fraction 2 yielded methyl esters of fatty acids and free fatty alcohols, characterized by means of GLC and mass spectroscopy as described before[11,12]. Further on, this record shows the presence of nonacosan-10-one as an individual peak, identified by mass spectrum: M^+ 422 ($C_{29}H_{58}O$), *m/z* 155, 170, 171, 295, 310, 311. Afte transesterification fraction 3 afforded methyl esters of hydroxyacids (according to GC-MS), along with methyl esters and free alcohol portion, both of n-series. The most significant alcoholic component of this fraction was nonacosan-10-ol (40 mg); M^+ 424 ($C_{29}H_{60}O$), *m/z* 139, 157, 279, 297, 406 [M−18, base peak].

After recrystallisation (hexane) the fraction 4 yielded the *ent*-isoalantolactone (*VIII*), m.p. 107—109°C, $[\alpha]_D^{22}$ −155° (*c* 0·22; $CHCl_3$); according to GLC 99·1% pure. The isoalantolactone (ref.[23]) is characterized by its m.p. 111—113°C, $[\alpha]_D$ +172°C ($CHCl_3$). Mother liquors contained (according to GC-MS) little amounts of a further lactone $C_{15}H_{20}O_2$, a ketone $C_{15}H_{22}O$ and alcohols $C_{15}H_{24}O$, resp. $C_{15}H_{26}O$.

Methods and Apparatus

TLC and column chromatography were done on silica gel. Hexane, tetrachloromethane and chloroform were used as solvents. GLC analyses were carried out on PYE series 104 Chromatograph, Model 24, provided with a dual column and detector (FI) system. Columns of 0.4 × × 150 cm size were filled with 3% SE-30 on Gas Chrom P (100—120 mesh). Alternative packings were 3% Carbowax 20 M on Chromosorb G, 20% Apiezon L on Chromosorb W, and 10%

DEGS on Chromosorb W. The linear relationship between the logarithm of retention time and the number of carbon atoms[24] was used for identification of homologous series. Preparative GC was performed on the same apparatus with the preparative adapter on SE-30 or Apiezon L. IR spectra were recorded on spectrometers UR 20 Zeiss and Perkin–Elmer (models 621 and 580). Mass spectra were done in AEI 902 apparatus either by direct inlet or in connection with the PYE series 104 Chromatograph, Model 64; ion source temperature 250°C, 70 eV.

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