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# Preliminary data on essential oil composition of the moss *Rhodobryum ontariense* (Kindb.) Kindb.

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**ABSTRACT** – The essential oil composition of the moss *Rhodobryum ontariense* obtained by hydrodistillation was preliminary analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Thirteen compounds (86.41% of the total oil) were identified. The main chemical constituents were phytol (31.95%) and 1-octen-3-ol (15.44%). Such an abundance of phytol has not yet been reported in other mosses. Four chemicals (*cis*-decahydronaphthalene, *allo*-hedycaryol, jasmone and 2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid, methyl ester) are first records, not only for the genus *Rhodobryum* and mosses in general, but also for the group of bryophytes as a whole.

**Mosses / *Rhodobryum ontariense* / Serbia / Phytochemistry / Essential oil**

## INTRODUCTION

Bryophyte chemistry is still poorly known, and the results on this issue are very scattered (Asakawa, 2007). To date, ca. 5% of total bryophytes have been chemically studied (Sabovljevic & Sabovljevic, 2008). Despite the fact that mosses are more numerous than liverworts, in chemical terms much less attention has been devoted to them (Karunen, 1974; Mues, 2000; Nillsson & Mårtensson, 1971; Suire *et al.*, 2000). Additionally, a limited number of mosses has been ever studied regarding essential oils, (Özdemir *et al.*, 2009; Zhao *et al.*, 1998), among which only a few European moss species (Saritas *et al.*, 2001).

Özdemir *et al.* (2009) investigated the essential oils of *Brachythecium salebrosum* (Hoffm. *ex* F. Weber *et* D. Mohr) Schimp., *Eurhynchiastrum pulchellum* (Hedw.) Ignatov *et* Huttunen and *Plagiomnium undulatum* (Hedw.) T. J. Kop. On the other side, Zhao *et al.* (1998) reported the volatile constituents

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of three mosses, *Entodon concinnus* (De Not.) Par., *Abietinella abietina* (Hedw.) M. Fleisch. and *Ptilium crista-castrensis* (Hedw.) De Not. The essential oils of mosses of the genera *Mnium*, *Plagiomnium*, *Homalia*, *Plagiothecium* and *Taxiphyllum*, collected in various forests from Germany, Austria, France and Italy, have been published by Saritas *et al.* (2001).

Finally, the volatile components of the moss *Rhodobryum giganteum* (Schwaegr.) Par., a counterpart of *Rhodobryum ontariense* (Kindb.) Kindb. not present in Europe, have been also identified (Li & Zhao, 2009). They found some aliphatic and aromatic aldehydes, monoterpene hydrocarbons and a sesquiterpene (α-farnesene), with 1-methoxy-2-propyl acetate and *n*-hexanal to be the most abundant compounds. Qiao *et al.* (2004) identified in *R. giganteum* hexadecanoic acid (62.80%) and hexahydrofarnesyl acetone (7.35%) as the main components.

Although there are chemical studies on *Rhodobryum* mosses namely, *R. giganteum* and *R. roseum* (Hedw.) Limpr., data on the chemistry of the moss *R. ontariense* are still lacking. The objective of this study was to identify volatile constituents as a contribution to the chemistry of this species.

## MATERIALS AND METHODS

### *Plant material*

The moss sample, collected in Serbia (the Deliblato Sands, NE of Belgrade) in November 2008, was air-dried and stored for one day in dark place at room temperature (25 ± 2ºC) before hydrodistillation. A voucher specimen has been deposited in the Herbarium of the Institute of Botany, University of Belgrade, Serbia (bryophyte collection BEOU No. 4708). Nomenclature follows Hill *et al.* (2006).

### *Isolation of the essential oil*

Air-dried green leaves of gametophyte tips (5 g) were used for the hydrodistillation. They were ground and steam distilled in a Clevenger apparatus for about two hours to obtain a colorless to pale yellow oil (yield: 0.23%).

### *Analysis of the essential oil*

Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) analyses were performed using an Agilent 7890A GC equipped with inert 5975C XL EI/CI MSD and FID detector connected by capillary flow technology 2-way splitter with make-up. A HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The GC oven temperature was programmed from 60°C to 300°C at a rate of 3°C/min and hold for 10 min. Helium was used as the carrier gas at 16.255 psi (constant pressure mode). The sample was analyzed in the splitless mode. The injection volume was 1 μL. GC detector temperature was 300°C. MS data was acquired in EI mode with scan range 30-550 *m/z*, source temperature 230°C, and quadrupole temperature 150°C; solvent delay was 3 minutes.

The components were identified based of their retention index and comparison with reference spectra (Wiley and NIST databases) as well as by retention time lock (RTL) method and RTL Adams data base. The retention indices were experimentally determined using the standard method (Van Den Dool & Kratz, 1963) involving retention times of *n*-alkanes, injected after the essential oil under the same chromatographic conditions. The percentage (relative) of the identified compounds was computed from their GC peak area. The quantitative composition of oil was GC (FID) analysed by internal normalization assuming an identical mass response factor for all compounds. In this study, only those components present in the oils in amounts higher than 0.1% were taken into consideration.

## RESULTS

The yield of essential oil isolated by hydrodistillation of the moss *Rhodobryum ontariense* was found to be 0.23% (w/w) based on dried material. The constituents identified by GC and GC/MS analysis, including two unidentified compounds, and the area percentages are summarized on Table I. Thirteen

Table 1. Chemical composition of *Rhodobryum ontariense* essential oil

| *Compound* | *RI*[a] | *Content,%* |
|---|---|---|
| Phytol | 2080 | 31.95 |
| 1-Octen-3-ol | 954 | 15.44 |
| -Ca | 1964 | 11.82 |
| α-Pinene | 904 | 11.55 |
| *n*-Nonanal | 1081 | 7.28 |
| *trans*-Verbenol | 1121 | 4.53 |
| *cis*-Decahydronaphthalene | 1074 | 3.78 |
| α-Muurolol | 1619 | 2.68 |
| 6,10,14-Trimethyl-2-pentadecanone | 1814 | 2.23 |
| -Cb | 1780 | 1.77 |
| *allo*-Hedycaryol | 1611 | 1.76 |
| Verbenone | 1189 | 1.55 |
| *n*-Decanal | 1186 | 1.31 |
| Jasmone | 1376 | 1.25 |
| 2,5,5,8a-Tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid, methyl ester | 1712 | 1.10 |
| Total | – | 100.00 |

[a]Retention index on HP-5MS and according to *n*-paraffins; Ca, Cb - not identified

compounds, accounting for 86.41% of the oil, were identified. The volatile components of *R. ontariense* included diterpene (phytol) and aliphatic (1-octen-3-ol) alcoholes, hydrocarbon and oxygenated monoterpenes (α-pinene, *trans*-verbenol and verbenone), sesquiterpenes (α-muurolol and *allo*-hedycaryol), aliphatic aldehydes (*n*-nonanal and *n*-decanal) and ketone (6,10,14-trimethyl-2-pentadecanone or hexahydrofarnesyl acetone), fatty acid derivative (jasmone), and bicyclic organic compounds (*cis*-decahydronaphthalene and 2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid, methyl ester). The components with molecular formula $C_{15}H_{24}O_2$ (11.82%, RT 46.092 min) and $C_{17}H_{26}O_2$ (1.77%, RT 39.646 min) could not be structurally identified by used databases.

## DISCUSSION

Four chemicals (*cis*-decahydronaphthalene, *allo*-hedycaryol, jasmone and 2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid, methyl ester) are first records, not only for the genus *Rhodobryum* and mosses in general, but also for the group of bryophytes as whole. On the contrary, 1-octen-3-ol, α-pinene, *n*-nonanal and *n*-decanal are very common constituents of the essential oils of moss species.

Most of previous review articles on the chemistry of mosses mentioned the absence or only scattered occurrence of terpenoid compounds, particularly sesquiterpene hydrocarbons (Asakawa, 1995; Huneck, 1983; Zinsmeister *et al.*, 1991). The first proof of the presence of a sesquiterpene hydrocarbon, ent-β-cedrene, together with a diterpene alcohol, dolabella-3,7-dien-18-ol, in the Japanese moss *Plagiomnium acutum* was reported by Toyota *et al.* (1998). *Allo*-hedycaryol, a germacrane alcohol, is one of two sesquiterpenes present in *R. ontariense* essential oil.

Phytol (3,7,11,15-tetramethyl-2-hexadecen-l-ol) was the major constituent of the oil. Such an abundance of this compound has not yet been reported in other mosses (Özdemir *et al.*, 2009). On the other side, the essential oil compositions of mosses known up to date usually express a greater diversity than reported here (Saritas *et al.*, 2001).

Comparison of the chemical constituents in the volatile oil of *R. ontariense* with corresponding ones of *R. giganteum* (Li & Zhao, 2009) shows that only three compounds are common (α-pinene, *n*-nonanal and *n*-decanal) which are quantitatively more present in the first mentioned moss species. However, they did not find phytol at all in their samples of *R. giganteum* from Yunnan Province (China). Further, Qiao *et al.* (2004) reported hexahydrofarnesyl acetone as the second main component in *R. giganteum* essential oil (7.35%); this compound is one of minor constituents in Serbian *R. ontariense* (2.23%). Generally, essential oils are very variable and this can be the consequence of environment, i.e. locality factors as well as of the period when the plants have been collected (temperature, water regime or both, etc.). Therefore, more studies should be done for better understanding *R. ontariense* volatile chemistry. The next steps are collecting the moss in different time points throughout two or three consecutive years and examining the chemical compounds in its essential oil related to collecting time.

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