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An insight into fatty acid chemistry of *Rhytididelphus squarrosus* (Hedw.) Warnst.

Boris Pejin[1*], Ljubodrag Vujisić[2], Aneta Sabovljević[3], Marko Sabovljević[3], Vele Tešević[1] and Vlatka Vajs[2]

1 Department of Organic Chemistry, Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

2 Center of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia

3 Institute of Botany and Garden, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

ABSTRACT: The fatty acid composition of the moss *Rhytididelphus squarrosus* (Hedw.) Warnst. (Hylocomiaceae) collected in Germany during winter time was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Nine fatty acids were identified in its chloroform:methanol extract 1:1: arachidonic acid (30.7%), α-linolenic acid (19.1%), linoleic acid (15.1%), *cis*-5,8,11,14,17-eicosapentaenoic acid (14.4%), palmitic acid (11.9%), *cis*-8,11,14-eicosatrienoic acid (4.1%), oleic acid (2.3%), γ-linolenic acid (1.4%) and stearic acid (1.0%). The results indicate that this plant species can be a good source of arachidonic acid collected during the winter.

Key words: moss; *Rhytididelphus squarrosus*; fatty acids; GC FID; GC-MS.

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INTRODUCTION

Bryophytes (mosses, liverworts and hornworts) are the second biggest group of land plants after the flowering plants with approximately 28.000 species spread worldwide. Although the chemical structure and occurrence of fatty acids and their derivatives in bryophytes have been reviewed by Huneck (1969), their chemistry is still poorly known with information on this being very scattered (Asakawa 2007). Partly, this is due to difficulties in collecting large quantities of pure material of a certain species. The most abundant fatty acids of these plants are also common to most of other organisms. However, typical for many of them is a high content of long-chain polyunsaturated fatty acids, particularly arachidonic acid and *cis*-5,8,11,14,17-eicosapentaenoic acid, compounds which are not found very abundantly in the rest of the plant kingdom (Gellerman *et al*. 1975). Indeed, the occurrence of these polyunsaturated fatty acids constitutes an outstanding compositional difference between mosses and other higher plants. On the other hand, it is known that these fatty acids are constituents of membranes in animal tissues which are essential for many physiological processes. Arachidonic acid is a precursor of the biologically active prostaglandins and leukotrienes (Marx 1982).

As part of our ongoing research on moss chemistry (Pejin *et al.* 2010, 2011a, 2011b, 2011c), a phytochemical investigation was conducted on the pleurocarp moss *Rhytididelphus squarrosus* (Hedw.) Warnst. (Hylocomiaceae). Two reports on fatty acids from the moss *R. squarrosus* have been published to date (Guschina & Harwood 2000, 2002). The most abundant fatty acid of this species collected during the summer near Cardiff, UK

*correspondence: borispejin@yahoo.com

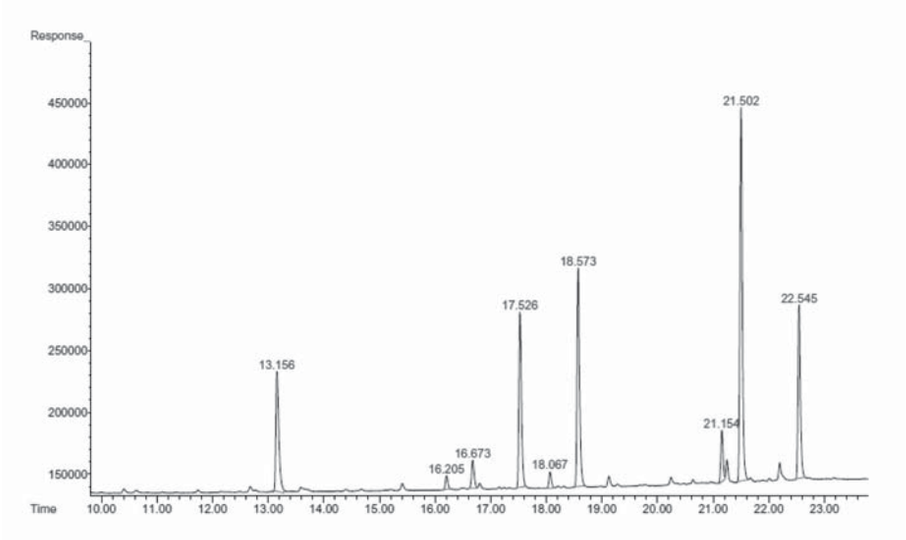

Fig. 1. Fatty acid methyl ester chromatogram for *R. squarrosus*; palmitic acid (*RT* 13.156 min); stearic acid (*RT* 16.205 min); oleic acid (*RT* 16.673 min); linoleic acid (*RT* 17.526 min); γ-linolenic acid (*RT* 18.067 min); α-linolenic acid (*RT* 18.573 min); *cis*-8,11,14-eicosatrienoic acid (*RT* 21.154 min); arachidonic acid (*RT* 21.502 min); *cis*-5,8,11,14,17-eicosapentaenoic acid (*RT* 22.545 min).

(July and September, 1998) from both lead-contaminated and non-contaminated areas was arachidonic acid (28.6% and 24.8%, respectively) (Guschina & Harwood 2002).

The objective of this study was to identify the pattern of fatty acids of the same bryophyte species during the winter time as a contribution to its chemistry.

MATERIAL AND METHODS

R. squarrosus was collected in Königforst near Köln (Germany) in December 2007. A voucher specimen was deposited in the Herbarium of the Institute of Botany, University of Belgrade, Serbia (bryophyte collection - BEOU No. 4707).

The moss was carefully selected and cleaned from soil and other contaminants. The gametophyte tips were used for the extraction. Air-dried parts of the sample were ground (1g) and extracted three times with chloroform:methanol 1:1 for 1h at room temperature (9.88%, extract yield). The extract was evaporated to dryness and trans-esterified with 5% H_2SO_4 in MeOH (v/v) for 4h at 80°C. The resulting methyl esters of fatty acids were analysed by comparing their GC FID chromatograms with that of a standard mixture (Supelco 37) obtained under the same conditions, and/or by analysis of GC-MS data using NIST 5 and Wiley 7 libraries.

GC analysis was performed on an Agilent 7890A GC system equipped with 5975C MSD and FID detectors, using a DB-23 column (30 m × 0.25 mm × 0.25 μm). Injection volume was 1 μL and injector temperature was 220°C with a 10:1 split ratio. Carrier gas (He) flow rate was 0.9 ml/min while the column temperature was linearly programmed in a range of 150-240°C at a rate of 4°C/min and then held at 240°C for 10 min. The transfer line was heated at 240°C. Three assay replicates and three biological replicates were analysed. The FID detector temperature was 300°C. EI mass spectra (70 eV) were acquired in the m/z range 40-500.

RESULTS

Nine fatty acids were identified in the chloroform:methanol 1:1 extract: arachidonic acid (30.7%), α-linolenic acid (19.1%), linoleic acid (15.1%), *cis*-5,8,11,14,17-eicosapentaenoic acid (14.4%), palmitic acid (11.9%), *cis*-8,11,14-eicosatrienoic acid (4.1%), oleic acid (2.3%), γ-linolenic acid (1.4%) and stearic acid (1.0%) (Fig.1).

These results for the composition and proportion of fatty acids are in good agreement with the previous report (Guschina & Harwood 2002), particularly regarding the content of arachidonic acid (Fig.2); the main difference is in presence of *cis*-13,16,19-docosatrienoic acid which was detected only in the earlier study (2.1%). Seasonal- and habitat- related changes in the fatty acid composition of various bryophytes exist and these vary from species to species (Karunen 1990). The major fatty acid of *R. squarrosus* seems to be arachidonic acid regardless of place and time of its collection. However, our sample collected during the winter had slightly more arachidonic acid (30.7%) in comparison with those collected in July (28.6%) and September (24.8%) as reported by Guschina & Harwood (2002). Since, the plants were not from the same habitats, in addition to time of the year, this variation could also be due to habitat differences.

Fig. 2. Arachidonic acid

Guschina & Harwood (2002) identified 17 fatty acids, 7 of which were in trace amounts which is in accordance with the 9 fatty acids recorded in this study.

For a general consideration of the seasonal-related changes in the fatty acid composition of this moss species, more replicate samples would need to be included in a follow-up study, including *in vitro* establishment of this moss.

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REZIME

Osvrt na hemiju masnih kiselina *Rhytididelphus squarrosus* (Hedw.) Warnst.

Boris Pejin, Ljubodrag Vujisić, Aneta Sabovljević, Marko Sabovljević, Vele Tešević, Vlatka Vajs

Sastav viših masnih kiselina mahovine *Rhytididelphus squarrosus* (Hedw.) Warnst. (Hylocomiaceae), sakupljene u Nemačkoj za vreme zimskog doba, preliminarno je ispitivan GC i GC-MS analizom. U njenom ekstraktu hloroform:metanol 1:1 identifikovano je devet viših masnih kiselina: arahidonska kiselina (30.7%), α-linolenska kiselina (19.1%), linolna kiselina (15.1%), *cis*-5,8,11,14,17-eikosapentaenska kiselina (14.4%), palmitinska kiselina (11.9%), *cis*-8,11,14-eikosatrienska kiselina (4.1%), oleinska kiselina (2.3%), γ-linolna kiselina (1.4%) i stearinska kiselina (1.0%). Dobijeni rezultati ukazuju da je ova biljna vrsta dobar izvor arahidonske kiseline u ispitivanom vremenskom periodu.

Ključne reči: mahovina; *Rhytididelphus squarrosus* (Hedw.) Warnst; masne kiseline; GC FID; GC-MS.