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# Free and Bound Volatiles of Garlic Mustard *(Alliaria petiolata)*

**Ivica Blažević* and Josip Mastelić**

*Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split, N. Tesle 10/V, 21000 Split, Croatia*

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Forty four volatile from *Alliaria petiolata* were identified after hydrodistillation in Clevenger-type apparatus. Essential oils were isolated from fresh, fresh autolyzed and dry plant material. Volatile compounds were analysed by gas chromatography (GC) and gas chromatography – mass spectrometry (GC-MS). The main components were organic nitrile and sulphur compounds. They were allyl isothiocyanate (40.3–47.2 %), 3,4-epithiobutane nitrile (3.8–10.2 %), allyl nitrile (0.6–7.6 %), allyl thiocyanate (1.2–2.1 %), that are released from sinigrin glucosinolate degradation. In oils from autolyzed plant material we found diallyl disulphide (7.2 %), diallyl sulphide (0.7 %), 3-vinyl-3,4-dihydro-1,2-dithiin (0.5 %) and 2-vinyl-4H-1,3-dithiin (0.3 %) that are released by degradation of *S*-alke(en)yl cysteine sulphoxide. Oils, except above mentioned volatiles, contain compounds without nitrogen and sulphur: phytol (4.0–26.3 %), palmitic acid (0–14.7 %), (*Z*)-hex-3-en-1-ol (0.4–6.2 %), nonanal (0–3.0 %), phenylacetaldehyde (0–2.8 %), *β*-ionone (0.3–1.9 %), 4-vinyl-2-methoxy-phenol (0.2–1.6 %), benzaldehyde (0.2–1.0 %). *O*-Glycosides with volatile aglycones were isolated and purified by »flash« chromatography. After *O*-glycoside hydrolysis by *β*-glucosidase from almonds, fourteen bound aglycons were identified for the first time in this plant. The main aglycones were: 2-phenylethanol (20.8 %), benzyl alcohol (16.7 %), eugenol (15.7 %), (*Z*)-hex-3-en-1-ol (4.8 %), 3-oxo-7,8-dihydro-α-ionol (4.7 %), methyl salicylate (4.6 %) and butane-2,3-diol (4.5 %).

*Keywords*
*Alliaria petiolata*
garlic mustard
*Brassicaceae*
glucosinolates
*O*-glycosides
allyl disulphide
volatile aglycones
isothiocyanates
GC-MS

## INTRODUCTION

Garlic mustard or Hedge garlic (*Alliaria petiolata* (M. Bieb.) Cavara et Grande) belongs to the *Brassicaceae* family. The leaves, flowers and fruit are edible as food for humans. They have a mild flavour of both garlic and mustard. The green seed-pods of *A. petiolata* are fried, the crushed seed is a condiment, and the garlic-scented leaves are added to savoury dishes. It is plant well known for their pharmacological properties. *A. petiolata* leaf tea is a blood purifier; the flowering plant is expectorant, antiseptic, stimulant, and anti-asthmatic, expels worms, and help heal wounds; a poultice treats skin ulcers and cuts, and juice stimulates blood flow.[1]

In its native range in Europe, at least 69 insects and seven fungi utilize Garlic Mustard as a food plant. In North America was introduced as a culinary herb in the 1860s, but because there are no significant natural enemies it is able to out-compete and displace many of their native species with its high seed productivity and monopolization of resources and is considered an invasive specie.[2]

* Author to whom correspondence should be addressed. (E-mail: ivica.blazevic@ktf-split.hr)

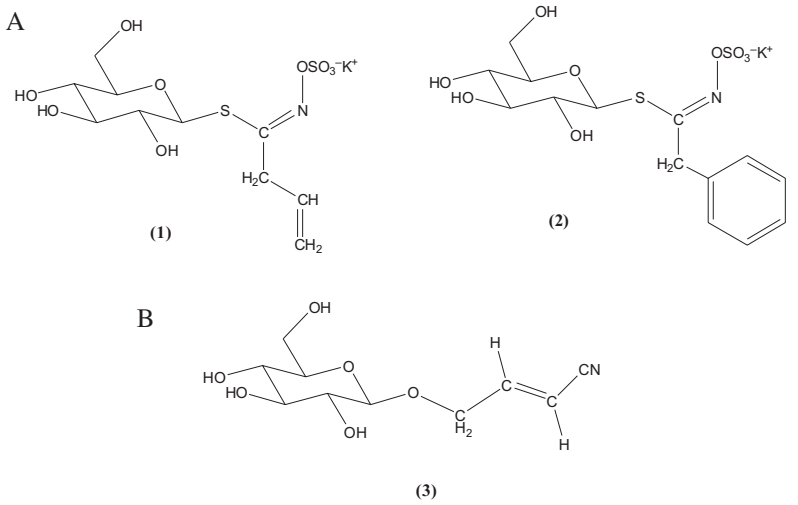


Figure 1. Glucosides with volatile aglycones found in *Alliaria petiolata*: A. Allyl glucosinolate (*sinigrin*) (**1**); Benzyl glucosinolate (*glucotropaeolin*) (**2**); B. (2*Z*)-4-(*β*-D-glucopyranosyloxy)-2-butenenitrile (*alliarinoside*) (**3**).

Characteristic flavour and odour of all *Brassicaceae* plants (cabbage, cauliflower, broccoli, radish, horseradish, mustard, oil rapeseed) has been attributed to volatile sulphur containing compounds that are developed through thioglucosidase hydrolysis of glucosinolates following tissue damage. It was found out that of glucosinolate compound class *Alliaria petiolata* contains sinigrin and glucotropaeolin (Figure 1A).[3] Although the primary biological function of glucosinolates in plants is unknown, glucosinolate breakdown products such as isothiocyanates, organic cyanides, oxazolidinethiones, epithionitriles and ionic thiocyanate ($SCN^-$) are proposed to act as allelochemicals and to play a role in plant defences against herbivores, pests, and pathogens and thus reduce the need for application of synthetic organic pesticides. Depending on glucosinolate composition and on the prevalence of hydrolysis products, consumption of glucosinolates by mammals has been linked with goitrogenic effects (5-vinyloxazolidin-2-thiones, thiocyanates) or with a reduced risk of developing cancer (isothiocyanates) in experimental animals. Natural isothiocyanates derived from aromatic and aliphatic glucosinolates are effective chemoprotective agents that block chemical carcinogenesis and prevent several types of cancer in rodent models.[4,5]

Haribal *et al.* reported (2*Z*)-4-(*β*-D-glucopyranosyloxy)-2-butenenitrile (*alliarinoside*), an ubiquitous non-cyanogenic nitrile glucoside in garlic mustard, that being *β*-glucoside probably hydrolyze in acidic solutions and enzymatically by *β*-glucosidases (Figure 1B).[6] *O*-Glycosidically bound volatile compounds (monoterpenes, norisoprenoids, aliphatics, phenols, benzene derivatives) have been reported in many plants.[7] These *O*-glycosides and glucosinolates were recently investigated in *Iberis sempervirens* L. (*Brassicaceae*).[8]

The objective of this work was to identify free and bound (*O*-glycosides and glucosinolates) volatile compounds of garlic mustard growing wild in Croatia.

## EXPERIMENTAL

### *Reagents*

Solvents and *β*-glucosidase were purchased from Fluka Chemie, Buchs, Switzerland. Octyl-*β*-D-glucopyranoside, silica gel for column chromatography (Kiesegel 60, 0.040-0.063 mm), pre-coated silica plates (Kiesegel 60, thickness 0.2 mm) for thin layer chromatography, ammonia and sodium sulphate were obtained from Merck, Darmstadt, Germany.

### *Plant Material*

Garlic mustard (*Alliaria petiolata*) plants were collected in the submediterranean region of south Croatia (near Sinj) during spring 2006 from wild-growing populations. The collection of plant material was during flowering in spring. The sample of plant material consisted of stems (*ca.* 15 cm), with leaves and flowers. Part of plant material was dried in a shaded place at room temperature for fifteen days. The voucher specimens are deposited at Department of Organic Chemistry, Faculty of Chemistry and Technology, Split, Croatia.

### *Preparation of Hydrolysis Products*

*Isolation of Volatiles*. – The volatiles were isolated from fresh (200 g) and dried plant material (100 g) by hydrodistillation in Clevenger type apparatus for 3 hours using pentane/ether (volume ratio, $\Psi$ = 1:1) for trapping. After distillation, the pentane/ether extract was separated and dried over anhydrous sodium sulphate. The extract was concentrated by carefully fractional distillation to a small volume (*ca.* 3 mL) and 1 μL of this solution was used for each GC and GC-MS analyses.

*Autolysis and Volatiles Isolation*. – Fresh collected plant material of *A. petiolata* was investigated for the presence of glucosinolates by detection of their autolysis products. 250 g of plant material was crushed in coffee mill and mixed with distilled water 800 mL, and left for autolysis 36 hours at 27 ± 2 °C. After natural autolysis, plant material was submitted for 3 h, to hydrodistillation of volatiles in Clevenger type apparatus. After distillation pentane/ether layer was separated, dried over $Na_2SO_4$ and concentrated. 1μL of this solution was used for GC and GC-MS analysis.

O-*Glycosides Isolation.* – A hundred grams of fresh plant material was submitted for 20 min to a boiling water (400 mL) to inactivate enzymes and simultaneously to extract water soluble compounds and partially remove volatile compounds by evaporation. Isolation by extraction and chromatographic purification was done as described in previous paper.[8] The obtained glycosidic fraction was concentrated to dryness and dissolved in a citrate buffer (pH = 5.5; 5 mL). The aqueous solution was washed with 5 × 5 mL of pentane-dichloromethane $\Psi$ = 2:1 and with 10 × 5 mL of pure pentane to remove the possibly existing hydrophobic compounds.

O-*Glycosides Hydrolysis*. – In a typical experiment, *β*-glucosidase from almonds (20 mg, 5-8 U/mg) was added to the glycosidic solutions along with 3 mL pentane for trapping the liberated aglycones. The hydrolysis was carried out for 72 h at 30 °C with the mixture being shaken occasionally. After hydrolysis, the pentane layer was separated and remaining aglycones were extracted from the aqueous layer with pentane (10 × 2 mL). The combined pentane extract was dried ($Na_2SO_4$) and concentrated to final volume of 0.5 mL, and 1 μL was used for GC and GC-MS analysis.

*Gas Chromatography (GC-FID)*

Gas chromatography analysis was performed on a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with flame ionisation detector and capillary column HP-101 (Methyl silicone fluid), 25 m × 0.2 mm i.d., coating thickness 0.2 μm. Chromatographic conditions were as follows: helium as carrier gas at 1.0 mL/min; injector and detector temperatures, 250 °C and 300 °C. Oven temperature was isothermal at 70 °C for 2 min, then increased to 200 °C, at a rate of 3 °C $min^{-1}$ and held isothermal for 15 min. Volume injected 1 μL. Split ratio 1:50.

*Gas Chromatography-Mass Spectrometry Analysis (GC-MS)*

Analysis was performed on a GC-MS Hewlett-Packard (model 5890 with a mass selective detector model 5971A, Hewlett Packard, Vienna, Austria) using two columns with different polarity of stationary phases. GC operating conditions: column HP-20M (Carbowax 20M, Hewlett Packard, Vienna, Austria), 50 m × 0.2 mm i.d., film thickness 0.2 μm; column temperature programmed from 70 °C isothermal for 4 min, to 180 °C at a rate of 4 °C $min^{-1}$; column HP-101 (Dimethylpolysiloxane, Hewlett Packard, Vienna, Austria), 25 m × 0.2 mm i.d., film thickness 0.2 μm; column temperature programmed from 70 °C isothermal for 2 min, to 200 °C at a rate of 3 °C $min^{-1}$; carrier gas: helium; flow rate: 1 mL $min^{-1}$; injector temperature: 250 °C; volume injected: 1 μL; split ratio: 1:50. MS conditions: ionization voltage: 70 eV; ion source temperature: 280 °C; mass range: 35–300 mass units.

*Identification and Quantitative Determination of Components*

The individual peaks were identified by comparison of their retention indices to those of authentic samples, as well as by comparing their mass spectra with the Wiley 6.0 library (Wiley, New York) and NIST98 (National Institute of Standards and Technology, Gaithersburg) mass spectral database and literature.[9,10]

The percentage composition of the samples was computed from the GC peak areas using the normalization method (without correction factors). For quantification of total volatiles was used gravimetric method (after removing solvents by careful fractional distillation). The content of *O*-aglycones was estimated from the GC-peak areas related to the GC-peak area of 1-octanol (liberated from octyl-*β*-D-glucoside as internal standard for *O*-glycosides). Preliminary GC-MS analysis showed the absence of 1-octanol as potential aglycone. The yields of volatiles were expressed as mg/kg respecting the masses of starting fresh or air-dried plant material. The component percentages were calculated as the mean value of component percentages on column HP-20M and HP-101 from duplicate analysis.

## RESULTS AND DISCUSSION

Isolated volatile compounds from garlic mustard prior and after autolysis, as well as *O*-aglycones obtained by hydrolysis with *β*-glucosidase from *O*-glycosides were subjected to a detailed GC and GC-MS analysis.

Table I. shows yields and chemical composition of volatiles. It is generally known that yields and chemical composition of volatile compounds depend prior of species and subspecies of the plant (genetic factors). Furthermore the same plant in different conditions such as type of soil, climatic conditions, stage of growth and other factors can give different yield and chemical composition of volatile compounds. The yields of volatiles obtained from fresh plant material by Clevenger method were 138.0 mg/kg (without autolysis) and 280.3 mg/kg (with autolysis), and yield from dried plant material was 197.1 mg/kg of dried plant material. Differences in volatile yields were due to enzymatic and thermal degradations of non-volatile precursors, that are present in *Alliaria petiolata*. Usage of different plant materials enables to conclude about these precursors present in the plant.

Hydrodistillation in Clevenger type apparatus insures isolation and concentration of the free volatile compounds and volatiles obtained by enzymatic and thermal degradation of non-volatile precursors during isolation. Twenty six were identified from oil isolated from fresh plant material, thirty three compounds were identified in

TABLE I. Volatile compounds from garlic mustard (*Alliaria petiolata*)

| Identified compounds | Peak area 1(a) % | 1(b) % | 1(c) % | Mode of identification |
|---|---|---|---|---|
| diallyl sulphide(f) | – | 0.7 | – | $I_1$, $I_2$, MS |
| (*E*)-hex-2-en-1-al(h) | 0.9 | 0.7 | 0.8 | $I_1$, $I_2$, MS |
| butane-2,3-diol(h) | 0.2 | 0.3 | 0.7 | $I_1$, $I_2$, MS |
| 2-furfural(h) | – | – | 0.5 | $I_1$, $I_2$, MS |
| (*Z*)-hex-3-en-1-ol(h) | 6.2 | 4.7 | 0.4 | $I_1$, $I_2$, MS |
| allyl isothiocyanate(d) | 47.2 | 45.1 | 40.3 | $I_1$, $I_2$, MS |
| benzaldehyde(h) | 0.2 | 1.0 | 0.8 | $I_1$, $I_2$, MS |
| 2-pentylfuran(i) | 0.2 | – | 0.5 | $I_1$, $I_2$, MS |
| (*Z*)-hex-3-en-1-yl acetate(g) | 0.3 | – | – | $I_1$, –, MS |
| (*E,E*)- hepta-2,4-dien-1-al(h) | 0.1 | – | 0.4 | $I_1$, $I_2$, MS |
| phenylacetaldehyde(h) | 0.6 | – | 2.8 | $I_1$, $I_2$, MS |
| diallyl disulphide(f) | 0.3 | 7.2 | 0.6 | $I_1$, $I_2$, MS |
| nonanal(h) | 0.8 | – | 3.0 | $I_1$, $I_2$, MS |
| benzyl alcohol(i) | – | 0.1 | – | $I_1$, $I_2$, MS |
| 2-phenylethyl alcohol(i) | – | 0.2 | 0.2 | $I_1$, $I_2$, MS |
| phenylacetonitrile(b) | 0.6 | 0.7 | 2.2 | $I_1$, $I_2$, MS |
| 3-vinyl-3,4-dihydro-1,2-dithiin(f) | – | 0.5 | – | $I_1$, $I_2$, MS |
| methyl salicylate(i) | 0.1 | 0.7 | 0.1 | $I_1$, $I_2$, MS |
| 2-vinyl-4H-1,3-dithiin(f) | – | 0.3 | – | $I_1$, $I_2$, MS |
| $\beta$-cyclocitral(i) | 0.1 | 0.1 | tr | $I_1$, $I_2$, MS |
| (*Z*)- hex-3-en-1-yl valerate(g) | 0.1 | – | – | $I_1$, –, MS |
| octanoic acid (*caprylic acid*)(g) | – | 0.8 | – | $I_1$, $I_2$, MS |
| 2*H*-1-Benzopyran(i) | 0.3 | – | 0.4 | $I_1$, $I_2$, MS |
| benzyl isothiocyanate(d) | 0.2 | 0.2 | – | $I_1$, $I_2$, MS |
| 4-vinyl-2-methoxy-phenol(i) | 0.2 | 0.3 | 1.6 | $I_1$, $I_2$, MS |
| 4-vinylphenol(i) | – | 0.3 | – | $I_1$, $I_2$, MS |
| 1*H*-indole(i) | – | – | 0.2 | $I_1$, $I_2$, MS |
| $\beta$-ionone* (i) | 0.5 | 0.3 | 1.9 | $I_1$, $I_2$, MS |
| decanoic acid (*capric acid*)(g) | – | 0.1 | – | $I_1$, $I_2$, MS |
| undecanoic acid(g) | – | 0.2 | – | $I_1$, –, MS |
| dodecanoic acid (*lauric acid*)(g) | – | 0.3 | – | $I_1$, –, MS |
| tetradecanoic acid (*myristic acid*)(g) | – | 0.5 | – | $I_1$, –, MS |
| dibutylphtalate(g) | – | – | 0.8 | $I_1$, $I_2$, MS |
| methyl palmitate(g) | – | 0.6 | – | $I_1$, $I_2$, MS |
| pentadecanoic acid(g) | – | 0.5 | – | $I_1$, –, MS |
| methyl linoleolate(g) | 0.4 | – | – | $I_1$, $I_2$, MS |
| ethyl palmitate(g) | 0.3 | 0.2 | 0.3 | $I_1$, $I_2$, MS |
| hexadecanoic acid (*palmitic acid*)(g) | – | 14.7 | 1.2 | $I_1$, –, MS |
| phytol*(h) | 22.6 | 4.0 | 26.3 | $I_1$, –, MS |
| allyl nitrile(e) | 7.6 | 0.6 | 6.6 | –, $I_2$, MS |
| (*Z*)- pent-2-en-1-ol(h) | – | 0.2 | – | –, $I_2$, MS |
| allyl thiocyanate(f) | 1.2 | 2.1 | 1.8 | –, $I_2$, MS |
| 3,4-epithiobutane nitrile(e) | 7.1 | 10.2 | 3.8 | –, $I_2$, MS |
| vitispirane(i) | 0.1 | 0.4 | tr | –, $I_2$, MS |
| Total identified | 98.5 | 98.9 | 98.3 | |
| Number of identified comp. | 26 | 33 | 27 | |
| (d)Isothiocyanates; No. (%) | 2(47.4) | 2(45.3) | 1(40.3) | |
| (e)Nitriles and epithionitriles; No. (%) | 3(15.3) | 3(11.5) | 3(12.6) | |
| (f)Other sulphur compounds; No. (%) | 2(1.5) | 5(10.8) | 2(2.4) | |
| (g)Fatty acids and esters; No. (%) | 4(1.1) | 9(17.9) | 3(2.3) | |
| (h)Aliphatic alcohols and carbonyl comp.; No. (%) | 8(31.6) | 6(10.9) | 9(35.7) | |
| (i)Other compounds; No. (%) | 7(1.5) | 8(2.4) | 9(4.9) | |
| Yield / (mg/kg) | 138.0 | 280.3 | 197.1 | |

Plant material and isolation methods
(a) fresh plant material – Clevenger method;
(b) fresh plant material-autolysis – Clevenger method;
(c) dried plant material – Clevenger method

Identification methods
$I_1$: retention indicies on HP-101; $I_2$: retention indices on HP-20M; MS: mass spectra; tr: trace (< 0.1 %); –: not detected; *: correct isomer is not identified

oil isolated from grinded plant material after autolysis and twenty seven in oil isolated from dried plant material. The compounds are listed in order of their elution on the HP-101 column. Identification compounds of total oil were 98.3–98.9 %.

Vaughn *et al.* reported that allyl isothiocyanate (20.4 %) and benzyl isothiocyanate (35.6 %) were the major phytotoxic volatile hydrolysis products of glucosinolates found in dichloromethane extract of the garlic mustard tissues.[11] The major volatile in oils obtained by hydrodistillation in our experiments from all samples was allyl isothiocyanate (40.3–47.2 %). It is formed by sinigrin degradation into corresponding unstable aglucon intermediate and Lossen rearrangement. Other degradation products, from the same glucosinolate, 3,4-epithiobutane nitrile (3.8–10.2 %), allyl nitrile (0.6–7.6 %) and allyl thiocyanate (1.2–2.1 %) were also detected among volatiles in all samples. It is now accepted that epithiospecifier protein is responsible for nitrile formation although the actual mechanism is still an open question. Epithiospecifier protein uses the unstable thiohydroximate generated by myrosinase to produce nitriles and epithionitriles.[5] There are various theories for thiocyanate formation such as an enzyme that rearranges the glucosinolate, an isomerase that acts on the isothiocyanate and factors which act on the aglycone to generate thiocyanate.[12] According to Luthy and Benn[13] only three glucosinolates – allyl, benzyl and 4-methylthiobutyl – can produce organic thiocyanates because only these three have R groups that are capable of forming stable intermediary cations. Burow *et al*. investigated glucosinolate hydrolysis in *Lepidium sativum* and recently identified a thiocyanate-forming protein (TFP). They reported that TFP not only catalyze thiocyanate and simple nitrile formation from benzylglucosinolate, but also the formation of simple nitriles and epithionitriles from aliphatic glucosinolates.[14] Sheme degradation of sinigrin with corresponding structures of volatile compounds (found in volatile mixture) is given in Figure 2.

Benzyl isothiocyanate (0–0.2 %) and benzeneacetonitrile (0.6–2.2 %), degradation products of glucotropaeolin, were also found. Benzyl thiocyanate, that can be formed according to previous mentioned theory, was not detected among volatiles.

Diallyl disulphide (7.2 %), diallyl sulphide (0.7 %), 3-vinyl-3,4-dihydro-1,2-dithiin (0.5 %) and 2-vinyl-4H-1,3-dithiin (0.3 %) were other organosulphur compounds found in oil after autolysis. (Figure 3). Only diallyl disulphide was found among isolated volatiles from fresh and dry plant, but in smaller percentage (0.3–0.6 %).

This compound class is formed by chemical transformation of a series of volatile sulphur compounds generated of relatively stable, *S*-alk(en)yl cysteine sulphoxides, odourless flavour precursors, by the enzyme alliinase. These secondary metabolites are *S*-methyl cysteine sulphoxide

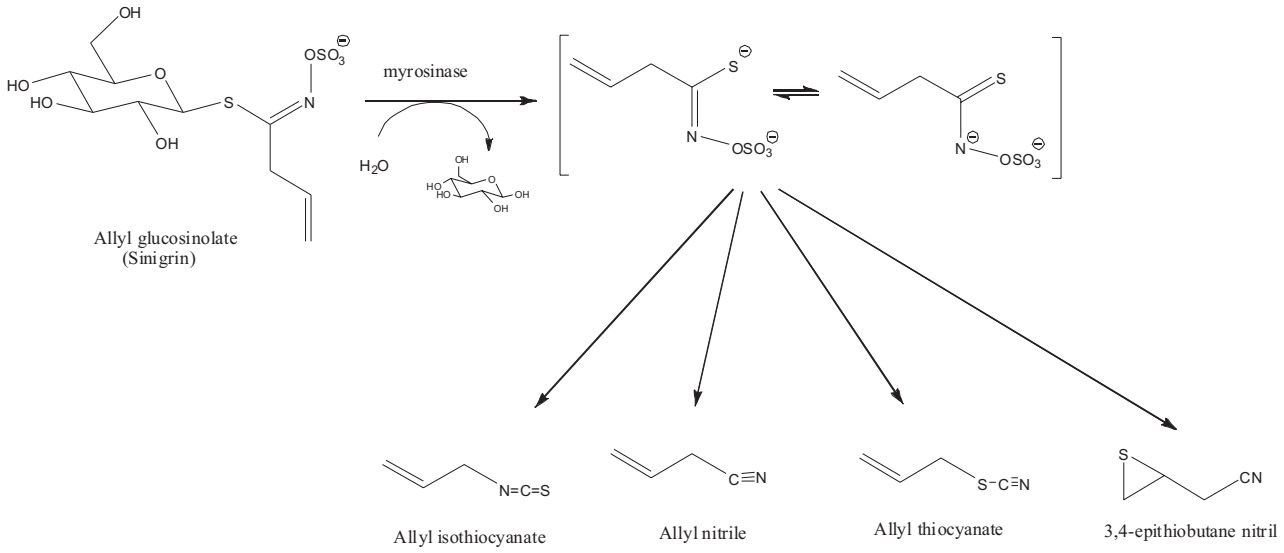


Figure 2. Degradation of sinigrin.

(methiin; present in most *Alliums*, some *Brassicaceae*), *S*-allyl cysteine sulphoxide (alliin, characteristic of garlic), *S-trans*-prop-1-enyl cysteine sulphoxide (isoalliin; characteristic of onion), and *S*-propyl cysteine sulphoxide (propiin; in onion and related species).[15] They are characteristic of *Allium* species and, as not being related to *Alliaceae*, it is suggested that *Alliaria petiolata* ability to synthesize these identical compounds probably developed independently.[16,17]

They are all odourless until the tissue is damaged, at which point they generate the volatile and reactive sulphur-containing chemicals. When garlic mustard is crushed, the enzyme *alliinase*, a C-S lyase, that is present in the vacuoles within the cell is released. The enzyme transforms *S*-alk(en)yl cysteine sulphoxide giving initially alk(en)yl sulphenic acids. These are highly reactive intermediates that immediately produce thiosulphinates by condensation reactions. Thiosulphinates are very unstable compounds and give rise to further rearrangements leading to a wide variety of derived sulphur compounds. Diallyl disulphide was found to be a major degradation compound of these class compounds when oil of *Alliaria petiolata* is obtained after autolysis and hydrodistillation in Clevenger type apparatus (Figure 3). These sulphur-containing compounds are very important because they are responsible for characteristic pungent aroma and taste, that garlic mustard is famous for. Also, it is believed they play key roles in many biological effects.[18–20]

Oils, except above discussed volatiles, content compounds without nitrogen and sulphur. The main volatiles found were: phytol (4.0–26.3 %), palmitic acid (0–14.7 %), (*Z*)-hex-3-en-1-ol (0.4–6.2 %), nonanal (0–3.0 %), phenylacetaldehyde (0–2.8 %), *β*-ionone (0.3–1.9 %), 4-vinyl-2-methoxy-phenol (0.2–1.6 %) and benzaldehyde (0.2–1.0 %).

(*Z*)-Hex-3-en-1-ol, butane-2,3-diol, methyl salicylate, benzyl alcohol, 2-phenylethanol, 4-vinylphenol and 4-vinyl-2-methoxy-phenol were identified among the volatile *O*-aglycones. Vitispiran (tr-0.4 %), that was found among volatiles of all oils, was first identified in grape juice, wines and vanilla beans and have since been found to occur as constituents of many aromas (raspberries, yellow passion fruit, black tea).

Octanoic, decanoic, undecanoic, dodecanoic, tetradecanoic, pentadecanoic and hexadecanoic acids and their esters were present in oil obtained after autolysis, while in other oils they were absent or present in small percentages. Aliphatic volatile compounds (alcohols, aldehydes, acids and their esters) can be originated of fatty acid catabolism.[21] *β*-Cyclocitral and *β*-ionone, that were present in garlic mustard, are originating from the degradation of carotenoids.

## O-*Aglycones*

Isolated and purified *O*-glycosides were hydrolysed by *β*-glucosidase, and liberated *O*-aglycones were analysed

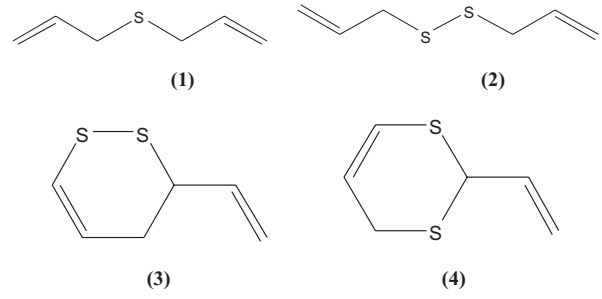


Figure 3. Some organosulphur compounds found among volatiles isolated from *A. petiollata*: (**1**) diallyl sulphide; (**2**) diallyl disulphide; (**3**) 3-vinyl-3,4-dihydro-1,2-dithiin and (**4**) 2-vinyl-4H-1,3-dithiin.

TABLE II. Volatile *O*-aglycones from garlic mustard (*Alliaria petiolata*)

| Identified compound | Peak area $\frac{1}{\%}$ [(d)] | Mode of identification |
|---|---|---|
| 1. (*Z*)-hex-3-en-1-ol | 4.8 | $I_1$, $I_2$, MS |
| 2. butane-2,3-diol | 4.5 | $I_1$, $I_2$, MS |
| 3. methyl salicylate | 4.6 | –, $I_2$, MS |
| 4. 2-methoxy-phenol | 0.4 | –, $I_2$, MS |
| 5. benzyl alcohol | 16.7 | $I_1$, $I_2$, MS |
| 6. 2-phenylethanol | 20.8 | $I_1$, $I_2$, MS |
| 7. phenol | 1.6 | $I_1$, $I_2$, MS |
| 8. 4-methyl-phenol (*p*-cresol) | 0.3 | –, $I_2$, MS |
| 9. eugenol | 15.7 | $I_1$, $I_2$, MS |
| 10. 4-vinyl-2-methoxy-phenol | 0.7 | $I_1$, $I_2$, MS |
| 11. 4-vinylphenol | 1.8 | –, $I_2$, MS |
| 12. 1*H*-indole | 1.8 | $I_1$, $I_2$, MS |
| 13. 3-oxo-α-ionol | 1.8 | $I_1$, $I_2$, MS |
| 14. 3-oxo-7,8-dihydro-α-ionol | 4.7 | –, $I_2$, MS |
| Total identified | 80.2 | |
| Yield / (mg/kg) | 25.3 | |

[(d)] volatiles obtained by *O*-glycosides hydrolysis from fresh plant material

$I_1$: retention indicies on HP-101M; $I_2$: retention indices on HP-20M; MS: mass spectra; – : not detected

by GC and GC-MS. The content of glycosidically bound volatile compounds in fresh plant material was 25.3 mg $kg^{-1}$. Fourteen aglycones were identified representing 80.2 % of the total volatile *O*-aglycones.

Aliphatic alcohols, derivatives of phenylpropanes and $C_{13}$ norisoprenoides were identified. The results are shown in Table II. The compounds are listed in order of their elution on the HP-20M column. The main aglycones were: 2-phenylethanol (20.8 %), benzyl alcohol (16.7 %), eugenol (15.7 %), (*Z*)-hex-3-en-1-ol (4.8 %), 3-oxo-7,8-dihydro-α-ionol (4.7 %), methyl salicylate (4.6 %) and butane-2,3-diol (4.5 %). Many of these compounds were identified as the most often aglycones in many plants. Comparing the chemical composition of *O*-aglycones (Table II) with the essential oil obtained after autolysis (Table I) seven identified compounds were identified, as previously mentioned.

Eugenol and other *p*-hydroxyphenylpropanes which were identified in many plants as main aglycones can be connected with lignin biosynthesis *via* peroxidase-hydrogen peroxide system according to Siegel.[22] The aliphatic volatiles (alcohols, carbonyls, acids) can originate from fatty acid catabolism, and aromatic volatiles (alcohols, acids, carbonyls) from cinnamic acid catabolism.[21]

## CONCLUSION

Investigation of *Alliaria petiolata* has uncovered many new compounds that are known in other plant families. The chemistry of this plant could provide new insight into the successful spread to the west of a garlic mustard weed from Eurasia. Naturally occurring compounds in spices such as, sulphur and/or nitrogen compounds, phenols, esters and glycosides are known to show biological activity. Flavour and medicinal properties which garlic mustard is known for, can now be better understood through different free and bound volatile products that previously were not reported.

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# SAŽETAK

## Slobodni i vezani hlapljivi spojevi češnjače (*Alliaria petiolata*)

**Ivica Blažević i Josip Mastelić**

Hidrodestilacijom, korištenjem aparature po Clevenger-u, izolirana su četrdeset i četiri hlapljiva spoja češnjače (*Alliaria petiolata*). Za istraživanje je upotrebljen svježi biljni materijal prije i poslije autolize te suhi biljni materijal. Izolirani hlapljivi spojevi su analizirani plinskom kromatografijom (GC-FID) i vezanim sustavom plinska kromatografija-spektrometrija mase (GC-MS). Glavni spojevi bili su sumporni i/ili dušikovi spojevi: alil-izotiocijanat (40.3–47.2 %), 3,4-epitiobutilnitril (3.8–10.2 %), alilnitril (0.6–7.6 %), alil-tiocijanat (1.2–2.1 %), koji potječu od raspada sinigrina (glukozinolat). Također, nakon hidrodestilacije autoliziranog biljnog materijala u aparaturi po Clevengeru identificirani su dialil disulfid (7.2 %), dialil-sulfid (0.7 %), 3-vinil-3,4-dihidro-1,2-ditiin (0.5 %) i vinil-4H-1,3-ditiin (0.3 %), koji nastaju djelovanjem enzima aliinaze na *S*-alke(en)il cistein sulfoksi-de. Ulja, osim razmatranih hlapljivih spojeva, sadrže spojeve bez sumpora i dušika. Glavni spojevi su: fitol (4.0–26.3 %), palmitinska kiselina (0–14.7 %), (*Z*)-heks-3-en-1-ol (0.4–6.2 %), nonan-1-al (0–3.0 %), fenil-acetaldehid (0–2.8 %), *β*-jonon (0.3–1.9 %), 4-vinil-2-metoksi-fenol (0.2–1.6 %) i benzaldehid (0.2–1.0 %). *O*-Glikozidi s vezanim hlapljivim spojevima su izolirani i pročišćeni »flash« kromatografijom. Nakon hidrolize *O*-glikozida s *β*-glukozidazom iz badema je po prvi put identificirano četrnaest vezanih hlapljivih spojeva. Glavni aglikoni su bili: 2-feniletanol (20.8 %), benzil-alkohol (16.7 %), eugenol (15.7 %), (*Z*)-heks-3-en-1-ol (4.8 %), 3-okso-7,8-dihidro-α-jonol (4.7 %), metil-salicilat (4.6 %) i butan-2,3-diol (4.5 %).