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Volatile components of *Plantago lanceolata* (*Plantaginaceae*)

by Françoise Fons[1], Sylvie Rapior[1], Annick Gargadennec[1], Claude Andary[1] and Jean-Marie Bessière[2]

(1) Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, 15 av. Charles Flahault, F-34060 Montpellier Cedex 2

(2) Laboratoire de Chimie Appliquée, Ecole Nationale Supérieure de Chimie, 8 rue de l'Ecole Normale, F-34296 Montpellier Cedex 5

Summary.- Fruits, leaves and scapes of wild *Plantago lanceolata* (ribwort) were investigated for volatile compounds by GC/MS. Leaves of young ribworts (35-day-old) cultured *in vitro* were also analysed. The volatile compound proportion corresponds to 0.05%, 0.03% and 0.001% of fresh weight for fruits, leaves and scapes, respectively. Thirty-five and twenty-six components were identified from fruits and leaves, respectively, while scapes contained only seven volatile components. The major constituents of fruits were oct-1-en-3-ol (24.9%), hexahydrofarnesylacetone (15.7%), vanillic acid (9.8%) and neophytadienes (>10%); leaves contained mainly oct-1-en-3-ol (41.1%), (E),4(3-oxo-2,6,6-trimethylcyclohex-2-en-1-yl)-3-buten-2-ol (15.6%), 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-7-oxabicyclo[4,1,0]heptan-3-ol (6.9%) and benzoïc acid (6.3%). Neophytadienes were mainly found in both scapes of wild ribworts and leaves of seedling cultures.

Résumé.- Les substances volatiles des fruits, des feuilles et des hampes florales du plantain lancéolé (*Plantago lanceolata*) sauvage ont été analysées par chromatographie en phase gazeuse couplée à la spectrométrie de masse (CG-SM). Les feuilles des jeunes plantules cultivées *in vitro* (âgées de 35 jours) ont également été analysées. Le pourcentage de composés volatils dans chaque organe mesuré est de 0,05% de la masse fraîche pour les fruits, 0,03% de la masse fraîche pour les feuilles et de 0,001% de la masse fraîche pour les hampes florales. Trente-cinq et vingt-six composés volatils ont été respectivement identifiés dans les fruits et dans les feuilles. Sept composés volatils ont été isolés dans les hampes florales. Les composés majoritaires retrouvés dans les fruits sont le oct-1-en-3-ol (24,9%), l'hexahydrofarnésylacétone (15,7%), l'acide vanillique (9,8%) et les néophytadiènes (>10%) ; les feuilles contiennent majoritairement du oct-1-en-3-ol (41,1%), du (E)-4(3-oxo-2,6,6-trimethylcyclohex-2-en-1-yl)-3-buten-2-ol (15,6%), du 6-(3-hydroxy-1-butenyl)-1,5,5-triméthyl-7-oxabicyclo[4,1,0]heptan-3-ol (6,9%) et de l'acide benzoïque (6,3%). Dans les hampes florales du plantain sauvage et dans les feuilles du plantain cultivé *in vitro*, on ne retrouve que les néophytadiènes.

Key-words : *Plantago lanceolata* – volatile components – oct-1-en-3-ol – hexahydrofarnesylacetone – vanillic acid.

I. INTRODUCTION

Plantago lanceolata (ribwort) was investigated for several components groups, such as iridoids, i.e., catalpol, aucubin, asperuloside and globularine (Luckner *et al.*, 1968; El-Nagaar and Beal, 1980; Handjieva and Saadi, 1991; Long *et al.*, 1995; Harborne *et al.*, 1997), flavonoids, i.e., apigenin, scutellarein, kaempferol and kercetol derivatives (Haznagy *et al.*, 1976; Grigorescu *et al.*, 1973; Harborne and Baxter, 1995), coumarins, i.e., aesculine and cichorine (Haznagy, 1970) and phenylpropanoids, i.e., plantamoside, verbascoside, lavandulifolioside, cistanoside F and isoverbascoside (Andary *et al.*, 1988; Harborne and Baxter, 1995; Murai *et al.*, 1995). In this paper, ribwort was investigated for volatile components. Different parts of wild plants (scapes, leaves, fruits) and the leaves of fresh and freeze-dried *in vitro* cultured seedlings were compared using Gas Chromatography-Mass Spectrometry (GC/MS).

II. MATERIAL AND METHODS

Wild plants: Fresh wild *Plantago lanceolata* L. was collected in the Faculty of Pharmacy of Montpellier in the summer of 1998. Leaves (60 g), scapes (30 g) and fruits (60 g) of *P. lanceolata* were cubed and extracted with diethyl ether (from 100 to 400 ml) for 12 hours. The filtered organic extracts were concentrated to a small volume under nitrogen stream and then used for Gas Chromatography-Mass Spectrometry (GC/MS) analysis.

***In vitro* cultured seedlings**: Seeds of *P. lanceolata* were cultured in Murashige and Skoog culture medium (MS) as previously reported (Fons, 1998; Fons *et al.*, 1998). 35 day-old-plant leaves (30 g and 10 g for fresh and freeze-dried materials, respectively) were mashed in a mortar and extracted with diethyl ether (300 and 100 ml, respectively). The filtered extracts were then used for GC/MS analysis.

GC/MS analyses: Analyses were realised on Hewlett-Packard GC/MS system. The chromatograph was fitted with a 25 m x 0.20 mm polydimethylsiloxane OPTIMA-1 (fused film thickness: 0.25 µm) silica capillary column. The carrier gas was helium with a flow rate of approx. 0.6 ml/min; the injector and detector temperatures were 200°C and 220°C, respectively. The column was temperature programmed as follows: 50°C-200°C (3°C/min). The mass spectra were recorded on a mass selective quadrupole-type detector of the HP 5970A class, using a potential of 70 eV for ionisation by electron impact. Volatile compounds were identified by their mass spectra and retention indices (Stenhagen *et al.*, 1976; Jennings and Shibamoto, 1980; Adams, 1989; McLafferty and Stauffer, 1989; Pacakova and Pelt, 1992). The volatile compounds of *P. lanceolata* were listed in Table 1 in order of their retention indices.

III. RESULTS AND DISCUSSION

The volatile compound proportion corresponds to 462 $\mu g.g^{-1}$ of fresh weight for fruits (0.05%), 342 $\mu g.g^{-1}$ of fresh weight for leaves (0.03%) and 13 $\mu g.g^{-1}$ of fresh weight for scapes (0.001%). Thirty-five and twenty-six volatile components were identified by GC/MS from fruits and leaves, respectively, while scapes contained only seven volatile components (Table 1). The major compounds of fruits were oct-1-en-3-ol (24.9%), hexahydrofarnesylacetone (15.7%), vanillic acid (9.8%) and neophytadienes (> 10%); leaves contained mainly oct-1-en-3-ol (41.1%), (E)-4(3-oxo-2,6,6-trimethylcyclohex-2-en-1-yl)-3-buten-2-ol (15.6%), 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-7-oxabicyclo[4,1,0]heptan-3-ol (6.9%) and benzoïc acid (6.3%). Neophytadienes (59%) were mainly found in scapes of wild ribworts with (Z)-hex-3-enyl acetate (18.4%) and oct-1-en-3-ol (16.4%).

The main flavour compound in both leaves and fruits of ribworts was C8-aliphatic alcohol, oct-1-en-3-ol (24.9% and 41.1% in fruits and leaves, respectively) with fungal odour

(Maga, 1981); another C8-aliphatic alcohol, octan-3-ol, with weakly nutty fungal scent (Fischer and Grosch, 1987) was also found in low amounts from both leaves and fruits.

Three C6-derivatives, (Z)-hex-3-enol (4.8% in leaves), hexanal (1.1% in fruits) and (Z)-hex-3-enal (0.3% in leaves) are well-known to develop the "green odour" (Hatanaka, 1993).

Eugenol, wildly used instead of cloves oil (The Merck Index, 1996), is a common volatile compound from plants such as *Hyacinthus, Jasminum, Rosa, Stachys* (Knudsen *et al.*, 1993; Pélissier *et al.*, 1998) and mushrooms (Rapior *et al.*, 1997). Eugenol was identified for the first time in leaves (2.6%) and fruits (1.3%) of *P. lanceolata*.

Phenyl-2-ethanol (1.4% in leaves) and phenylacetaldehyde (1.4% in fruits) are often used in flavors and perfume industry for their rose and hyacinth scents, respectively (The Merck Index, 1996).

(E)-4(3-oxo-2,6,6-trimethylcyclohex-2-en-1-yl)-3-buten-2-ol and 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-7-oxabicyclo[4,1,0]heptan-3-ol are two oxydated compounds from the ionone family.

Among the volatile components identified from wild *P. lanceolata*, the three major plant volatile groups reported by Knudsen *et al.* (1993) were identified in the three parts of wild ribworts, i.e., isoprenoids (neophytadienes, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-7-oxabicyclo[4,1,0]heptan-3-ol, hexahydrofarnesylacetone), benzenoids (eugenol, phenylacetaldehyde, phenyl-2-ethanol, hydroxymethoxyacetophenone...) and aliphatic compounds (hexanal, heptanal, octanol, nonanol...).

Large amounts of palmitic acid were found in scapes of wild plants (280 $\mu g.g^{-1}$); lower amounts were also detected in fruits (78 $\mu g.g^{-1}$) and leaves (65 $\mu g.g^{-1}$). Toluene (37 $\mu g.g^{-1}$ in leaves), (*o, m, p*)-xylenes (7 $\mu g.g^{-1}$ and 2 $\mu g.g^{-1}$ in fruits and leaves, respectively) and pentadecane (24 $\mu g.g^{-1}$ in fruits) were also found in wild ribworts but were removed from the table 1 because exogen origins were suspected.

Young seedlings *in vitro* cultured (35-day-old) revealed only neophytadienes in both fresh and freeze-dried leaves.

IV. CONCLUSION

This study highlighted for the first time the broad spectrum of volatile compounds from leaves and fruits of *P. lanceolata* (oct-1-en-3-ol, hexahydrofarnesylacetone, vanillic acid, hexanal, eugenol...).

The results also show that neophytadienes (identified in both wild and young cultured *P. lanceolata*) are the first volatile compounds synthesised in the earlier states of plant growth. It would be interesting to compare the volatile profile evolution of seedlings until flowering with the profile of wild plants to estimate the importance of culture conditions on volatile compounds pathway.

Table 1.- Percentage* of volatile compounds in fruits, leaves and scapes of wild *Plantago lanceolata.*
Tableau 1.- Pourcentage* des composés volatils dans les fruits, les feuilles et les hampes florales de *Plantago lanceolata* sauvage.

R I	Volatile compounds	Fruits	Leaves	Scapes
789	NI	0.1	0.2	
790	hexanal	1.1	0.1	
799	butyl acetate	0.6	0.2	
800	n-octane	1.2		
829	(Z)-hex-3-enal		0.3	
843	(Z)-hex-3-enol		4.8	
861	2-methylbutanoic acid	0.3		
867	3-methylbutanoic acid	0.1		
921	benzaldehyde	0.4		
924	(E)-hept-2-enal	1.1		
949	oct-1-en-3-one	0.5		
950	octan-3-ol (isomer)	0.2		
965	oct-1-en-3-ol	24.9	41.1	16.4
980	(Z)-hex-3-enyl acetate		3.9	18.5
988	octan-3-ol	0.1	0.6	
989	(E)-hex-3-enyl acetate		0.1	
995	hexanoic acid	0.4		
1002	phenylacetaldehyde	1.4	0.2	
1021	phenylmethanol	0.3	0.2	
1059	NI			0.6
1083	nonanal	1.5	0.6	
1108	phenyl-2-ethanol	0.1	1.4	
1210	benzoic acid	0.6	6.3	
1239	(E)-dec-2-enal	4.6		
1240	4-vinylphenol		0.04	
1256	(2E, 4Z)-deca-2,4-dienal	0.1		
1280	nonanoic acid	0.1		
1287	(2E, 4E)-deca-2,4-dienal	0.4		
1297	4-vinyl-2-methoxyphenol	0.2	0.4	
1355	eugenol	1.3	2.6	
1391	vanillin	0.3	0.2	1.4
1423	4-hydroxybenzaldehyde	0.02	0.04	
1487	2-hydroxy-6-methoxy acetophenone	4.1	1.6	
1490	2(4-hydroxyphenyl)ethanol	7.3	2.7	
1620	vanillic acid	9.8	5.2	
1622	NI	1.6	0.2	
1661	(E)-4(3-oxo-2,6,6-trimethylcyclohex-2-en-1-yl)-3-buten-2-ol	1.6	15.6	
1677	NI	1.4		
1698	6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-7-oxabicyclo[4,1,0]heptan-3-ol	0.2	6.9	4.2
1717	NI	1.3	0.8	
1783	myristic acid	1.4		
1833	hexahydrofarnesylacetone	15.7		
1838	14-methylidene-2,6,10-trimethylhexadecene**	4.9	1.9	30.3
1844	NI	1.6		
1856	(Z)-2,6,10-trimethylhexadeca-1,3-diene**	2.8	0.7	7.7
1870	(E)-2,6,10-trimethylhexadeca-1,3-diene**	4.4	1.2	21.0

* relative percentage of the volatile components based on the GC/MS chromatographic area
** neophytadiene isomers
R = Retention Indices; NI = Not Identified

BIBLIOGRAPHY

Adams R.P., 1989.- Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic Press, San Diego, 469p.

Andary C., M.E. Motte-Florac, A. Gargadennec, R. Wylde & A. Heitz, 1988.- Les esters caféiques du genre *Plantago*. Identification et valeur chimiotaxinomique. *Plantes Med. Phytother.*, **22**, 17-22.

El-Naggar, L.J. & J.L. Beal, 1980.- Iridoïds. A review. *J. Nat. Prod.*, **43**, 649-707.

Fischer K.H. & W. Grosch, 1987.- Volatile compounds of importance in the aroma of mushrooms (*Psalliota bispora*). *Lebensm.-Wiss. u.-Technol.*, **20**, 233-236.

Fons F., 1998.- Biotransformation de l'acide cinnamique par *Plantago lanceolata* cultivé *in vitro* - Accumulation du plantamoside et du verbascoside dans les plantes entières et les racines transformées par *Agrobacterium rhizogenes*. Thèse de Doctorat, Université Montpellier I, 220p.

Fons F., A. Gargadennec, A. Gueiffier, J.L. Roussel & C. Andary, 1998.- Effects of cinnamic acid on polyphenol production in *Plantago lanceolata*. *Phytochemistry*, **49**, 697-702.

Grigorescu E., U. Stanescu, V. Basceanu & M.M. Aur, 1973.- Phytochemical and microbiologic control of some plant species used in folk medicine II. *Plantago lanceolata* L., *Plantago media* L., *Plantago major* L. *Rev. Med. Chirurg.*, **77**, 835-841.

Handjieva N. & H. Saadi, 1991.- Iridoid glucosides from *Plantago altissima* L., *Plantago lanceolata* L., *Plantago atrata* Hoppe and *Plantago argentea* Chaix. *Z. für Naturforsch.*, **46**, 963-965.

Harborne J.B. & H. Baxter, 1995.- Phytochemical Dictionary. Taylor & Francis (eds), London, 791p.

Harborne J.B., H. Baxter & G.P. Moss, 1997.- Dictionary of Plant Toxins. J. Wiley & Sons (eds), Chichester, 523p.

Hatanaka A., 1993.- The biogeneration of green odour by green leaves. *Phytochemistry*, **34**, 1201-1218.

Haznagy A., 1970.- Recent results with plantaginis folium (Plantain leaves). *Herba Hung.*, **9**, 57-63.

Haznagy A., G. Toth & E. Bula, 1976.- Apigenin –7-O-monoglucosid im Kraut von *Plantago lanceolata*. *Pharmazie*, **31**, 482-483.

Jennings W. & T. Shibamoto, 1980.- Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillar Gas Chromatography. Academic Press, New York, 472p.

Knudsen J.T., L. Tollsten & L.G. Bergström, 1993.- Floral scents. A checklist of volatile compounds isolated by head-space techniques. *Phytochemistry*, **33**, 253-280.

Long C., C. Moulis, E. Stanislas & I. Fourasté, 1995.- L'aucuboside et le catalpol dans les feuilles de *Plantago lanceolata* L., *Plantago major* L. et *Plantago media* L. *J. Pharm. Belg.*, **50**, 484-488.

Luckner M., A. Haznagy, O. Bessler & E. Korn, 1968.- Vorschläge für den Drogenteil des DAB 7 - 37. Mitteilung: Herba Plantaginis. *Pharmazie*, **23**, 395-400.

Maga J.A., 1981.- Mushroom flavor. *J. Agric. Food Chem.*, **29**, 1-4.

McLafferty F.W. & D.B. Stauffer, 1989.- The Wiley NBS Registry of Mass Spectra Data. J. Wiley & Sons (eds), New York, 2087p.

Murai M., Y. Tamayama & S. Nishibe, 1995.- Phenylethanoids in the herb of *Plantago lanceolata* and inhibitory effect on arachidonic acid-induced mouse ear edema. *Planta Med.*, **61**, 479-480.

Pacakova V. & L. Pelt, 1992.- Chromatographic Retention Indices. E. Horwood (ed), New York, 218p.

Pélissier Y., C. Marion, S. Rapior & J.M. Bessière, 1998.- Volatile components of *Stachys corsica* Pers. (*Lamiaceae*). *J. Essent. Oil Res.* (in press).

Rapior S., C. Marion, Y. Pélissier & J.M. Bessière, 1997.- Volatile composition of fourteen species of fresh wild mushrooms (Boletales). *J. Essent. Oil Res.*, **9**, 231-234.

Stenhagen E., S. Abrahamsson & F.W. McLafferty, 1976.- Registry of Mass Spectral Data. J. Wiley & Sons (eds), New York, 1670p.

The Merck Index, 1996.- Twelfth edition, Merck & Co., Inc., White House Station, 1741p.