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COMPARATIVE STUDY OF VOLATILE COMPOUNDS IN THE FRESH FRUITS OF *Mandragora autumnalis*

L. O. Hanuš[*], *V. M. Dembitsky*, and *A. Moussaieff*

Department of Medicinal Chemistry and Natural Products, School of Pharmacy,
P.O. Box 12065, The Hebrew University of Jerusalem, Jerusalem 91120, Israel

SUMMARY

Extracts of *Mandragora autumnalis* (mandrake) fruit have been analyzed by GC–MS. One-hundred and thirty-five compounds were identified in extracts obtained from the fruit after removal of the seeds – twenty-three *n*-alkanes, one branched-chain alkane, two cyclohexanes, eight alkenes, two branched-chain alkenes, three alcohols, three aldehydes, six ketones, eight heterocyclic compounds, four thio compounds, six benzene hydrocarbons, three phenols, eighteen carboxylic acids, and forty-eight esters of carboxylic acids.

INTRODUCTION

Mandrake is the common name for members of the Mediterranean plant genus *Mandragora* which belongs to the nightshade family (Solanaceae). There are two species of *Mandragora*, *Mandragora officinalis* var. *vernalis* (*Mandragora officinarum*) with white flowers and *Mandragora officinalis* var. *autumnalis* (*Mandragora autumnalis*) with purple flowers. *Mandragora autumnalis* is a perennial herb with thick tuberous roots native to southern Europe, the Middle East, and North Africa [1,2]. Surprisingly, the chemistry of this mysterious plant has not yet been studied intensively [3].

For many centuries, mandragora was one of the most important medicinal plants and a herb of great cultural value. Its use throughout history is nothing less than fascinating. Although mandragora was once commonly thought to be the biblical dudaim, we doubt this famous biblical aphrodisiac is mandragora. [4]. It is beyond doubt, however, that mandragora had extraordinarily important value in ancient pharmacopoeias, and this has continued until recent times. Dioscorides wrote at length on the mandragora's medicinal qualities, mentioning the root, the fruit, and the

leaves as remedies for many and varied ailments, including pain, insomnia, eye diseases, inflammation, and ulcers. The mandrake was used as an aphrodisiac in post-biblical times and is mentioned in later literature, especially in medieval treatises [4]. Its use in folklore peaked during the Middle Ages, when it was believed to be a magical plant in European folklore. Its anthropomorphic root has played an extremely important role in European medieval magic and witchcraft. As we know today, its "power" was probably because of the alkaloids atropine, scopolamine, and hyoscyamine, toxic and potentially deadly tropane alkaloids that can lead to respiratory paralysis and even death.

Given mandrake's popular use in many different fields of medicine, one might assume much phytochemical and pharmacognostic work would have been performed on the plant. In fact, we found very little work has been performed on any active compounds from mandragora other than alkaloids. We took it upon ourselves to perform thorough analysis of mandrake fruit. Mandragora fruit is the only edible part of the plant and is still consumed in the Holy Land, especially by Bedouins.

The amounts of the tropane alkaloids atropine and scopolamine in different parts of *Mandragora autumnalis* from Morocco have been investigated by capillary gas chromatography. The highest levels of atropine (0.2%) were found in roots collected while the plant was flowering [5]. The odoriferous constituents of mandrake fruit (*Mandragora officinarum* L.) were first studied almost twenty years ago. By use of capillary GC–MS fifty-five compounds were identified. The major compounds among these were ethyl butyrate (22%), hexanol (9%), and hexyl acetate (7%). In addition, over 7% were odour-contributing sulphur-containing compounds [6]. Small concentrations of even-numbered γ-lactones, which contribute to the pleasant aroma, have been found in a dichloromethane extract of the completely ripe fruit [7]. In another study fresh ripe fruit of *Mandragora autumnalis* were analyzed by water distillation and the headspace compounds were analysed by GC–MS. The main compounds of the essential oil (100 compounds, comprising 92% of the oil, were characterized) were ethyl caprate (14.2%), ethyl laurate (13.1%), and decyl acetate (10.7%). The main headspace components (49 compounds, comprising 99.2% of the total, were identified) were ethyl caproate (34.6%) and ethyl butyrate (25.5%) [8]. The occurrence of volatile chemicals in aromatic plants is not only an indication of chemical diversity but may also help solve problems with the taxonomy of comprehensively studied genera [9]. The lipid composition of the seeds and fruit of *Mandragora turcomanica* has been com-

pared. The degree of saturation of the fatty acids of the triacylglycerides and phospholipids and of the free fatty acids of the fruit flesh is higher than those of the lipid classes from the seeds [10]. Many of the compounds identified contribute to different extents to pleasant aroma and taste of the fruits.

EXPERIMENTAL

Plant Material

Unripe and ripe fruit of *Mandragora autumnalis* were collected from plants in the surroundings of Jerusalem. There are two varieties of this plant, which can only be recognized during the flowering season (they have blue and/or light violet flowers of different shape). We collected both varieties. The weight of the fruit was between 3.55 and 47.55 g, mostly 20–30 g (Fig. 1).

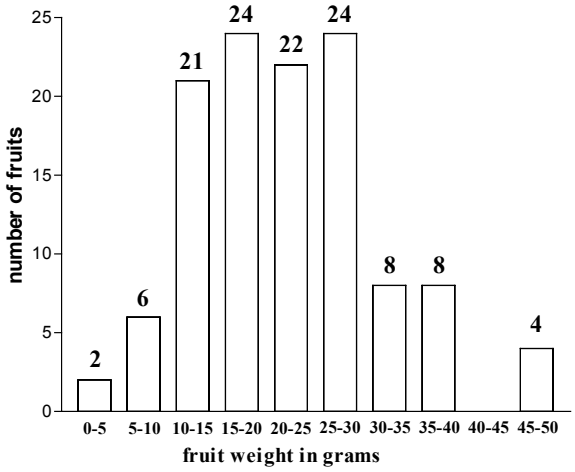

Fig. 1

Weight distribution of the fruit of *Mandragora autumnalis*

Extraction

The seeds were removed from the mandragora fruit. Flesh and peel constituted, on average, 84.7% of the total weight of fruit and seeds.

In the first experiment the peel and flesh of ripe mandragora fruit were liquefied in a glass mixer, and extracted with hexane and, subsequent-

ly, dichloromethane. In the second experiment the peel and flesh of both ripe and unripe fruit were liquefied in a glass mixer and lyophilized in a round-bottomed flask. After lyophilization the weight of the residue was 10.55% of the original weight for unripe fresh fruit and 7.39% for ripe fresh fruit.

Dry samples were then extracted as follows:

1. The first part was extracted with a mixture of ethanol–water (10 g was extracted with 100 mL ethanol plus 20 mL water overnight at RT).
2. The second part was extracted successively with petroleum ether, ethyl acetate, and methanol (150 mL solvent for 15 min at RT; the procedure was repeated three times for each solvent.

All extracts were then filtered by use of a vacuum filter (90 μm pore filter paper) and under gravity (150 μm pore filter paper) and the filtrate was evaporated by reduced-pressure rotary evaporation at 35°C.

GC–MS Analysis

Qualitative analysis of the samples was performed by GC–MS with a Hewlett–Packard G1800A GCD system comprising an HP-5971 gas chromatograph with electron ionization detection. Compounds were separated on an HP-5MS (Agilent Technologies) ultra-low-bleed 5% phenyl polydimethylsiloxane capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Two methods were used for analysis:

1. Splitless injection was performed with a purge time of 1.0 min. The carrier gas was helium at a flow rate of 1 mL min^{-1}. The column temperature was maintained at 40°C for 4.0 min then programmed at 5° min^{-1} to 80°C and then at 10° min^{-1} to 280°C. The inlet temperature was 250°C, the detector temperature 280°C, and the solvent delay 4 min.
2. Splitless injection was performed with a purge time of 1.0 min. The carrier gas was helium at a flow rate of 1 mL min^{-1}. The column temperature was maintained at 40°C for 4.0 min then programmed at 5° min^{-1} to 50°C and then at 30° min^{-1} to 280°C. The inlet temperature was 250°C, the detector temperature 280°C, and the solvent delay 5.20 min.

Libraries

The NIST/EPA/NIH and Wiley 7th Mass Spectral Libraries, and comparison with published spectral data were used for identification of the compounds.

RESULTS AND DISCUSSION

One-hundred and thirty-five compounds were identified by GC–MS of different extracts of unripe and ripe fruits of mandragora. In Table I we list the amounts (%) of the most important compounds found in the hexane extract. We compared the chemical composition of different extracts. To facilitate comparison, only the major components identified in each fraction are listed (with percentage content).

1(a) In the ethanol–water extract obtained from lyophilized, unripe fruit the main compounds were 34.3% ethyl linolenate, 14.2% ethyl palmitate, 10.6% ethyl stearate, 5.6% acetic acid, and 4.5% borneol.

1(b) In the ethanol–water extract of lyophilized ripe fruit the main compounds were 41.6% 5-hydroxymethyl-2-furancarboxaldehyde, 16.7% 3-methyl-2,5-furandione, 13.1% 2-furancarboxaldehyde, and 6.9% 5-methyl-2-furancarboxaldehyde.

In the ethanol extracts three ethyl esters only were identified – palmitate, linolenate, and stearate. Of these compounds only stearate was not found in non-ethanolic extracts.

2(a) In the petroleum ether extract of lyophilized unripe fruit the main compounds were 18.0% pimelic ketone, 5.9% butyl palmitate, 5.6% dodecane, 4.8% tetradecane, 4.1% tridecane, 4.1% undecane, 4.0% butyl stearate, 1.1% pentadecane, 0.9% decane, and 0.5% ethyl palmitate.

2(b) The petroleum ether extract of lyophilized ripe fruit differed from that of the unripe fruit, with the main components being 5.1% butyl caprate, 3.6% butyl laurate, 3.4% tetradecane, 3.4% vianol, 3.0% 1-octadecene, 2.0% eicosane, 1.9% pentadecane, 1.8% tricosane, 1.7% hexatriacontane, 1.6% pentacosane, 1.4% hexadecane, 1.2% tetracosane, 1.0% docosane, 0.9% heneicosane, 0.6% heptadecane, and 0.4% tridecane.

3(a) In the ethyl acetate extract of lyophilized unripe fruit the main compounds identified were 9.2% butyl butyrate, 7.1% 1-hexadecene, 6.2% palmitic acid, and 5.1% 1-octadecene.

3(b) In the ethyl acetate extract of lyophilized ripe fruit the main compounds were 43.2% ethyl 3-ethoxypropanoate, 15.2% cyclohexanone, 4.4% 3-hydroxy-2,4,4-trimethylpentyl ester of 2-methyl-propanoic acid, 2.0% 3-hexadecene, and 1.7% 1-octadecene.

4(a) In the methanol extract of lyophilized unripe fruit the main compounds were 81.4% 5-hydroxymethyl-2-furancarboxaldehyde, 3.8% acetic acid, 2.9% 2-furancarboxaldehyde, 2.8% 3-methyl-2,5-furandione,

Table I

Chemical components of extracts of *Mandragora autumnalis* fruits

Compound	Amount (%)	Compound	Amount (%)
Heptane	tr	Vianol	tr
Decane	tr	Eugenol*,**	1.03
Undecane	tr	Isoeugenol*	0.66
Dodecane	tr	Capric acid	tr
Tridecane**	tr	Stearic acid	tr
Tetradecane**	tr	Margaric acid	tr
Pentadecane**	0.11	Palmitic acid	1.68
Hexadecane	0.60	*n*-Pentadecanoic acid	tr
Heptadecane	tr	Myristic acid	0.40
Octadecane	tr	Lauric acid	1.62
Nonadecane	tr	Caprylic acid	tr
Eicosane	0.06	Acetic acid	0.64
Heneicosane	tr	Formic acid	tr
Docosane	0.69	Hydrocinnamic acid	tr
Tricosane	tr	Benzoic acid	tr
Tetracosane	tr	11-*cis*-Octadecenoic acid	tr
Pentacosane	tr	Oleic acid	1.89
Hexacosane	0.25	9-Hexadecenoic acid	tr
Heptacosane	tr	Linoleic acid	6.56
Octacosane	0.40	Cinnamic acid	0.50
Nonacosane	tr	Ethyl propanoate	tr
Hexatriacontane	0.09	Methyl stearate	tr
Tetratetracontane	tr	Ethyl stearate	tr
Farnesane	tr	Butyl stearate	tr
Cyclododecane	tr	Hexyl stearate	tr
Methylcyclohexane	tr	Methyl palmitate	tr
5-Methyl-1-undecene	tr	Ethyl palmitate	0.26
1-Pentadecene	tr	Butyl palmitate	tr
1-Hexadecene	tr	Ethyl myristate	0.30
3-Hexadecene	tr	Isopropyl myristate	0.34
7-Hexadecene	tr	Methyl laurate	0.26
1-Heptadecene	tr	Ethyl laurate*	3.19
5-Octadecene	tr	Butyl laurate	0.72
1-Nonadecene	tr	Methyl caprate	0.53
Squalene	0.64	Ethyl caprate	6.15
Neophytadiene	0.06	Butyl caprate	1.13
Borneol	tr	Ethyl caprylate	8.70
2-Methyl-1-hexadecanol	tr	Butyl caprylate	2.22
n-hexanol*,**	tr	Hexyl caprylate	1.21
Myristaldehyde	tr	Ethyl caproate	4.40
n-Nonylaldehyde	tr	Ethyl butanoate	tr
Benzaldehyde*,**	tr	Butyl butyrate*,**	1.83
Cyclohexanone	tr	*n*-Hexyl butyrate*,**	3.02
1-Hydroxy-2-propanone	tr	Ethyl propanoate	tr

Table I (continued)

Chemical components of extracts of *Mandragora autumnalis* fruits

Compound	Amount (%)	Compound	Amount (%)
1-Hydroxy-2-propanone acetate	tr	*n*-Hexyl acetate*,**	6.50
Cyclopent-2-en-1,4-dione	tr	Caprylyl acetate	0.95
β-Ionone**	0.13	Decyl acetate**	tr
Solavetivone	0.07	Benzyl acetate*,**	0.33
5-Hydroxymethyl-2-furan-carboxaldehyde	tr	Ethyl 3-hydroxy-butanoate	tr
3-Methyl-2,5-furandione	tr	Hexyl isovalerate	tr
2-Furancarboxaldehyde	tr	Hexyl 2-methylbutanoate	0.31
3-Furancarboxaldehyde	tr	Ethyl 3-ethoxypropanoate	tr
5-Methyl-2-furancarbox-aldehyde	tr	2-Methylpropanoic acid, 3-hydro-xy-2,4,4-trimethylpentyl ester	tr
2-Furanmethanol	tr	2-Methylpropanoic acid, 1-(1,1-dimethyl-ethyl)-2-methyl-1,3-propanediyl ester	tr
Benzaldehyde glyceryl acetal	tr	2-Methylpropanoic acid, 2,2-di-methyl-1-(2-hydroxy-1-methyl-ethyl)propyl ester	tr
δ-Laurolactone (δ-dodecalactone)	0.13	Benzyl benzoate	0.08
Methionol	tr	Butyl benzoate	tr
1,3-Oxathiane	tr	Ethyl dihydrocinnamate	tr
3-(Methylthio)propyl acetate	tr	Ethyl cinnamate*,**	0.60
Ethyl methylpropanethioate	1.34	Butyl cinnamate	0.54
Pseudocumene	tr	2-Ethylhexyl-*p*-methoxycinnamate	tr
2,5-Dimethyl-*p*-xylene	tr	Methyl oleate	tr
o-Ethyltoluene	tr	Ethyl oleate**	0.53
4-Ethyl-*o*-xylene	tr	Ethyl linolenate**	tr
1,3-Diethylbenzene	tr	Methyl linoleate	tr
p-Cymene**	tr	Ethyl linoleate**	tr

tr = trace (<0.05%)
*Previously found in *Mandragora officinarum* [6]
**Previously found in *Mandragora autumnalis* [8]

1.6% 5-methyl-2-furancarboxaldehyde, 0.6% palmitic acid, and 0.6% 2-furanmethanol.

4(b) In the methanol extract of lyophilized ripe fruit the main compounds identified were 57.0% 5-hydroxymethyl-2-furancarboxaldehyde, 11.7% 2-furancarboxaldehyde, and 9.5% 3-methyl-2,5-furandione. No methyl esters were identified in the methanol extracts.

5. In the hexane extract of unlyophilized ripe fruit the main compounds were 8.7% ethyl caprylate, 6.5% hexyl acetate, 6.2% ethyl caprate, 4.4% ethyl caproate, 3.2% ethyl laurate, and 3.0% hexyl butyrate.

6. In the dichloromethane extract of unlyophilized fresh ripe fruit the main compounds were 30.1% linoleic acid, 16.9% palmitic acid, 7.0 % squalene, 1.9% myristic acid, 1.8% ethyl caprate, and 1.4% ethyl laurate.

Many of the identified compounds contribute to different extents to pleasant aroma and taste of this fruit (because unripe fruit have no smell, the important "odour compounds" were present in ripe fruit only).

We find identification of solavetivone (0.07%) in the hexane extract of ripe fruit especially interesting. (−)-Solavetivone is a representative phytoalexin which has been isolated from potato tubers (*Solanum tuberosum*) infected with the blight fungus *phytophthora infestans* [11] and in aircured tobacco leaves (*Nicotiana tabaccum*) [12]. Solavetivone has previously been isolated from the Solanaceae species *Lycium chinense* [13], *Solanum melongena* [14], *Solanum aethiopicum* [15], and *Solanum jabrense* [16] only.

As far as we are aware this is the first time an attempt has been made to distinguish the ripe fruit of mandragora from the unripe fruit. The main compounds found in the ripe fruit and undetected in the unripe fruit are likely to be responsible for the fruit's special taste and odour and its so-called aphrodisiac qualities. We hope this comparison will lead to identification of novel flavour and odour compounds and be a step toward understanding the chemistry behind the mandragora myth. By comparing the chemical content of ripe and unripe fruit of *Mandragora automnalis*, we hope we have made the first step in identifying compounds likely to be of value to the food, cosmetics, and pharmaceutical industries.

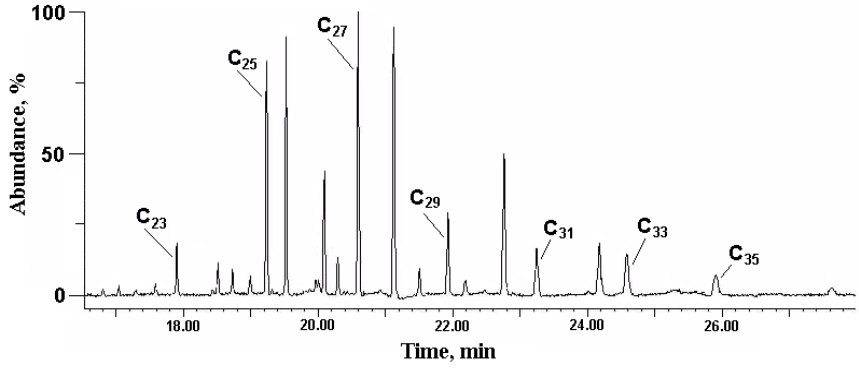

Fig. 2

TIC (total ion current) chromatogram obtained from the aliphatic *n*-hydrocarbons extracted from *Mandragora autumnalis* flower petals (hexane extract)

A typical TIC (total ion current) chromatogram obtained from *Mandragora autumnalis* flower petals (hexane extract), which have not previously been analysed, is presented in Fig. 2.

Despite its past popularity, no thorough research has been conducted on the phytochemistry of mandragora. Needless to say, no compounds of pharmacological value have been obtained from mandragora fruit (not taking into consideration the alkaloids from the seeds). We postulate that research on this plant, which has entered the "Hall of fame" of herbs of medicinal and cultural value, is not yet complete. We feel the many uses and popularity of mandragora in several cultures for many centuries justifies further research.

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