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Review

Substances isolated from *Mandragora* species

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Abstract

The present state of knowledge in the chemistry of mandragora plant is reviewed. Isolations and identifications of the compounds were done from all parts of this plant. Up-to-date more than 80 substances were identified in different species of the genus *Mandragora*.

Keywords: *Mandragora*; Mandrake; Solanaceae; Alkaloids; Natural substances

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Contents

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1. Introduction

Mandragora officinarum L. (mandrake) is one of the most ancient herbs known to mankind and yet remains one of the most misunderstood plants on earth (Vidal, 1982). The concept of this odd plant is surrounded with various myths and legends and recalls something mysterious, gloomy, and even horrible (Carter, 2003). Mandrake is among the long line of bewitching weeds, the most significant representative of magic power.

The intoxicating power of mandrake and its history goes back throughout the ages. Mandrake's fame struck all European civilizations. References to mandrake go back as far as the Holy Bible (Old Testament) and even further back in ancient eastern manuscripts. There was wide use of mandrake during the Greek and Roman

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ages, the medieval age as well as during the Renaissance period. This plant is known for its magic, aphrodisiac, healing, hallucinogenic, as well as poisonous properties. The fruits of the plant, also-called love apples, were believed to increase fertility. The value of mandrake as an aphrodisiac and as an aid to conception is mentioned in Genesis 30:14–17 and Song of Solomon 7:12–14, but some authors make it doubtful (Zohary, 1982).

The name *Mandragora* is derived from two Greek words implying "hurtful to cattle". The Arabs call it "Satan's apple". It was also valued in ancient Greece and Rome for its intoxicating and narcotic properties and was used as a surgical anesthetic. As early as in AD 93, the historian Josephus Flavius described the process of "dangerous" mandrake root collection. In the Roman Empire, it was considered dangerous to uproot a mandrake. The root of the mandrake resembles a phallus or a human body and for this reason was believed to have occult powers. It was "the female" root that was carved in the Middle Ages into a human shape. It was believed to bring good luck and wealth. Medieval witches were said to harvest the root at night beneath the gallows. The crushed root was supposed to have caused hallucinations followed by a death-like trance and sleep. The root was also said to cause insanity, and was believed to have been used in flying potions. The popularity of mandrake in Europe began to decline in the 16th century.

The Codex Neapolitanus is one of the oldest manuscripts in the tradition of Materia Medica, the leading pharmacological work of Greco-Roman times, written by the Greek surgeon Pedanius Dioscorides in the 1st century A.D. (Peduto, 2001; Barbaud, 1994). Dioscorides gave a detailed description of mandragora (mandrake) in the book "Greek Herbal". Over the ages, the mandrake has been endowed with a wonderful and mystical aura. In this book, Dioscorides used for the first time the word anesthesia as absence of sensation as we understand it today.

Among angiosperm families, the Solanaceae rank as one of the most important to human beings. Species of the family are used for food (e.g., the potato or the tomato), drugs (tobacco; deadly nightshade; mandrake; sources of commercial alkaloids) and as ornamentals (petunia; velvet tongue; or butterfly flower). The family is a medium-sized one with approximately 90 genera and 3000–4000 species. Members of the Solanaceae are extremely diverse; in terms of habit, ranging from trees to small annual herbs; in habitat, from deserts to the wettest tropical rain forests; and in morphology, with astounding variation in many characters of both flowers and fruits. Recent work using a variety of datasets derived from plastid DNA has revealed interesting phylogenetic patterns in the Solanaceae (Olmstead et al., 1999).

Six species of the genus *Mandragora* are founded from the Eurasian continent (Schultes, 1970). They are *M. acaulis*, *M. autumnalis*, *M. caulescens*, *M. officinarum*, *M. turcomanica*, and *M. vernalis*. Unfortunately, only two species (*M. officinarum* and *M. turcomanica*) were properly examined for their chemical content. Further two species (*M. vernalis* and *M. autumnalis*) were examined only cursory and last two (*M. acaulis* and *M. caulescens*) not at all.

Today mandrake is almost a forgotten plant. For more than 100 years, chemists were curious to solve "the secret" of the chemical principle of the "magic" root and the "apples" of love. It is surprising that such a famous plant has been so little studied for its content compounds.

2. Alkaloids

Natural raw materials containing atropine (**1**) and related compounds (Lounasmaa and Tamminen, 1993) had long been known for their complex effect (mydriatic effect, antagonization of blood pressure decrease and increased smooth muscles tonus, decreased secretion activity, e.g., salvia, gastric juice, etc.).

Partial synthesis of atropine (**1**) from its two basic components, i.e., basic alcohol tropine (**2**) and tropic acid (**3**), performed as early as in the eighties of 19th century stimulated the attempts to prepare more efficient analogs (Holmes, 1949). These aims mainly involved the so-called tropeins, i.e., esters of tropine obtained by hydrolysis of natural alkaloids with various acids. The highest mydriatic activity exhibited esters with aromatic hydroxy acids containing asymmetric carbon (it must possess *S*-configuration) in their molecules. Ester of tropine with mandelic acid (**4**) – homatropine (**5**) (Casy, 1975) is still of practical significance. As far as the modification of the basic moiety of the molecule is concerned, quarternization favourably influences the parasympatolytic effect and its mydriatic component. The corresponding quaternary salts, e.g., *N*-methylhomatropinium nitrate (**6**) or analogous *N*-methylhomatropinium bromide are used (Ramasastry, 1970) (see Fig. 1).

A similar effect to that of atropine (**1**) is also exhibited by another natural alkaloid scopolamine (**7**), ester of tropic acid (**3**) and alcohol scopine (**8**). At the end of the 19th century the development of mydriatics was influenced by the knowledge acquired when investigating local anesthetics. With the aim of obtaining compounds with a lower toxicity, for which the tropine moiety was considered responsible, many synthetic compounds were prepared (Gearien and Mede, 1974).

Atropine (**1**) is the main active alkaloid of numerous Solanaceae (Baytop and Nushet, 1983), as racemic mixture of (*R*)- and (*S*)-hyosciamine (**9**). Hyosciamine

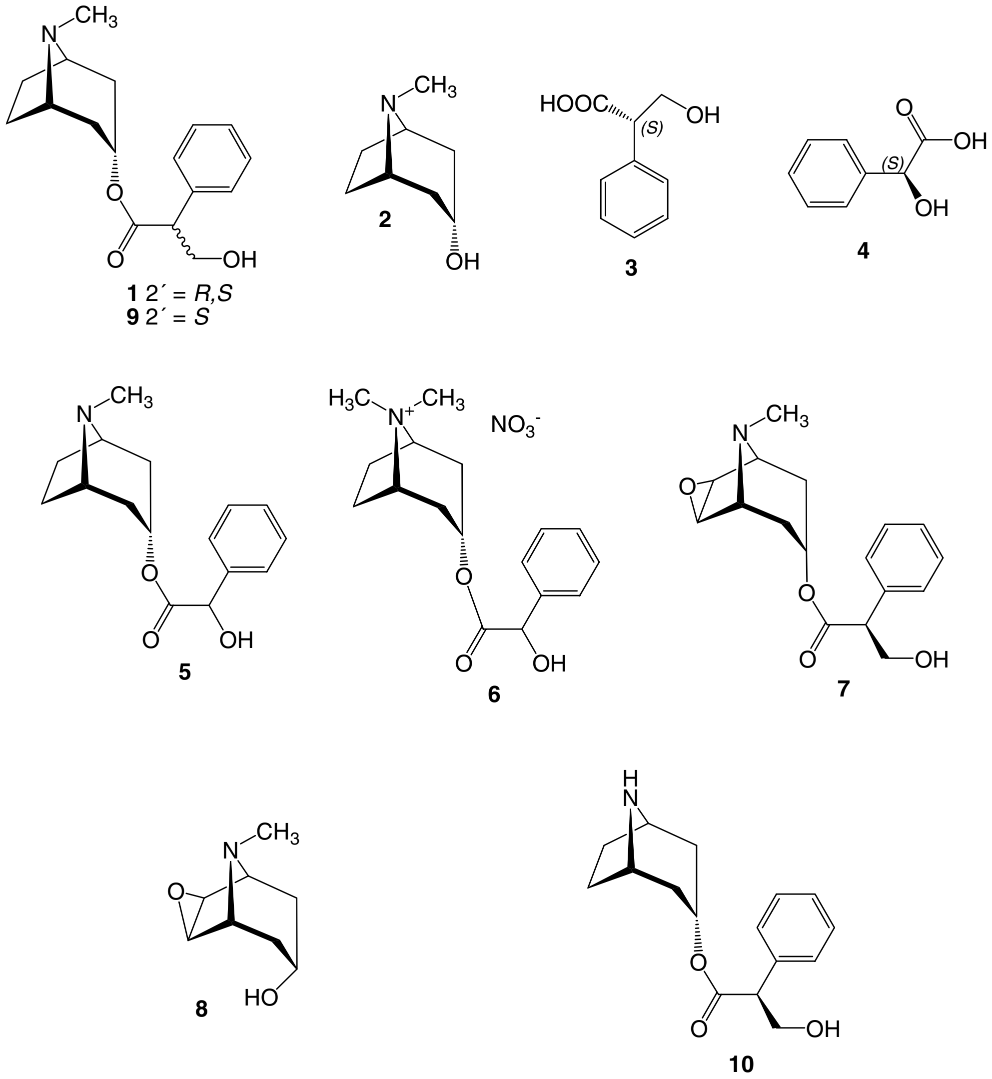


Fig. 1. Tropane alkaloids and hydroxy acids.

is present in fresh plants and with increasing time and mainly during extraction it readily racemizes to yield atropine. The general structure of tropic acid (**3**) and tropine ester was clarified by Willstätter who performed its total synthesis (Fodor, 1959). The absolute configuration was then determined in 1961 by the Fodor's group (Fodor, 1967). Tropic acid (**3**) is in (*S*)-configuration and the cyclopentane ring of tropine (**2**) is in envelope (C_s) form, whereas the piperidine ring is in chair form. The hydroxyl group of tropine is in *trans* with respect to *N*-CH_3. It contains two asymmetric centers (C-1 and C-5; the carbons C-1 and C-5 are chiral, but carbon C-3 is prochiral), four theoretically possible stereoisomers can thus be envisaged. In fact, only two exist since the methylamine bridge can connect C-1 and C-5 in *cis* only (i.e., C-1 and C-5 have (*S*) and (*R*) configuration (or vice versa)). Of the two optically active isomers, *S*, and *R*, *R* is most efficient. Although a synthetic preparation of atropine was described (by esterification of both components prepared synthetically), it is still primarily isolated from the natural material.

Ahrens (1889a,b) isolated the alkaloid mandragorin from the mandragora root, which was later proved to be a mixture of hyoscyamine (**9**) with a small amount of scopolamine (hyoscine) (**7**). Scopolamine is an ester of tropic acid and basic alcohol scopine (**8**). It differs from atropine (**1**) by the epoxide bridge between C(6) and C(7). The absolute configuration is analogous with atropine. The epoxide bridge is in *cis*-position with respect to *N*-CH_3. Scopolamine (**7**) is prepared exclusively from natural raw materials, usually from mother liquors after separation of atropine. Scopolamine is used similarly to atropine but is more efficient as mydriatic. In combination with analgesics of morphine type it is used for pre-surgical treatment.

The pioneering works of the German authors (Hesse, 1901; Thomas and Wentzel, 1898, 1901) from the break of the 19th and 20th century described the alkaloid content in mandragora in detail. Scopolamine (**7**) and hyoscyamine (**9**) with atropine (**1**) were isolated, although not in pure form.

Carr and Reynolds (1912) found in mandragora and other Solanaceae, norhyoscyamine (pseudohyoscyamine) (**10**).

One of the first chemical papers on the content of alkaloid compounds in *Mandragora officinarum* L., was published by Staub (1962). By means of paper chromatography and by preparation of crystalline picrates in mandragora roots he determined atropine (*R*,*S*-hyoscyamine) (**1**), scopolamine (**7**), cuscohygrine (**11**), scopine (**8**), and hyoscyamine (**9**). The presence of apoatropine (**12**) and belladonnines (**13**, **14**) was determined by paper chromatography only. Further, norhyoscyamine (**10**) was also detected in the roots (see Fig. 2).

Jackson and Berry (1973) identified in fresh and dry roots of *M. autumnalis* and *M. vernalis* (two species distinct from *M. officinarum*) scopolamine (**7**), hyoscyamine (**9**), cuscohygrine (**11**) and apoatropine (**12**). Belladonnines (**13**, **14**) were present in the dried roots, but

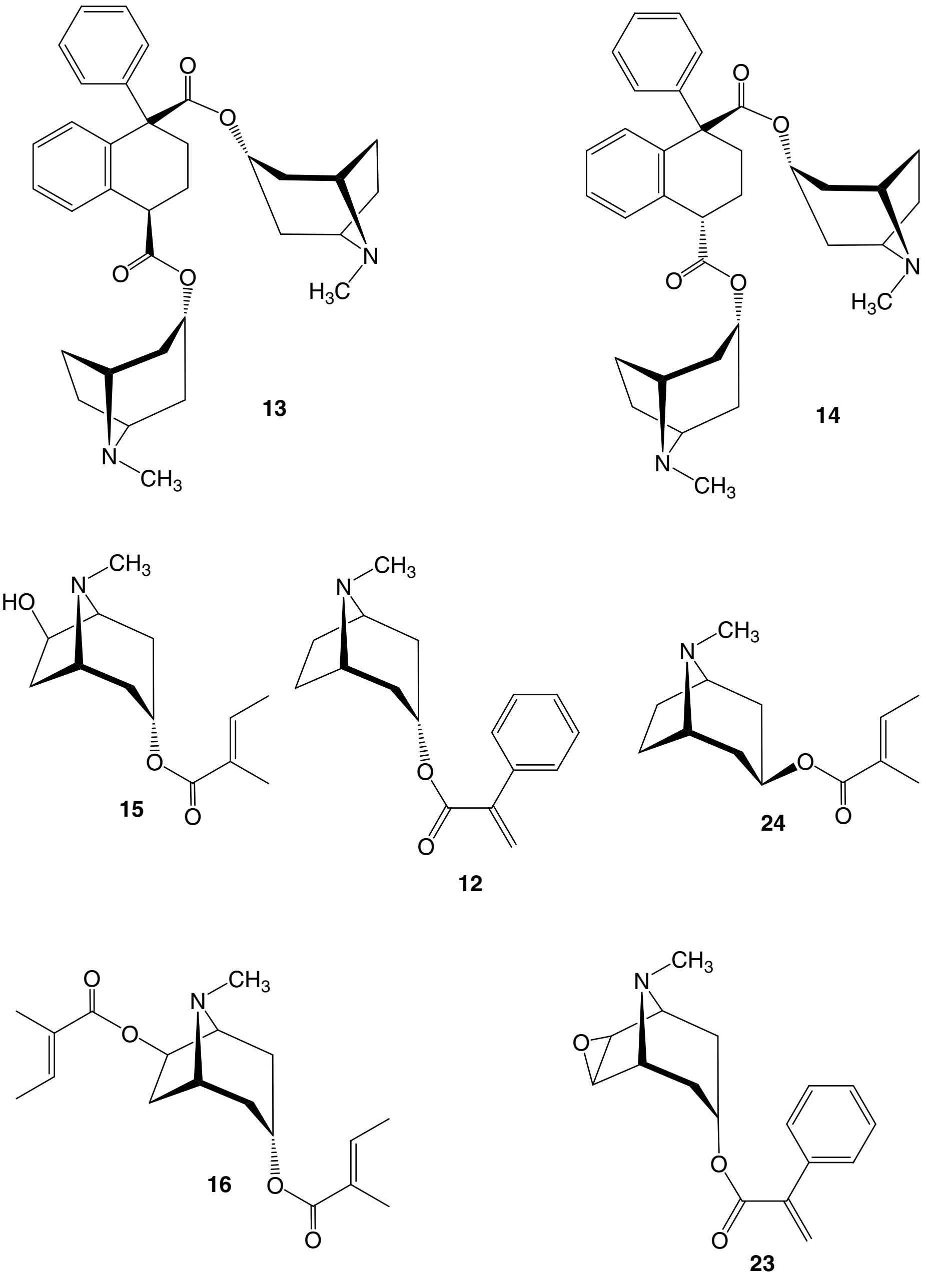
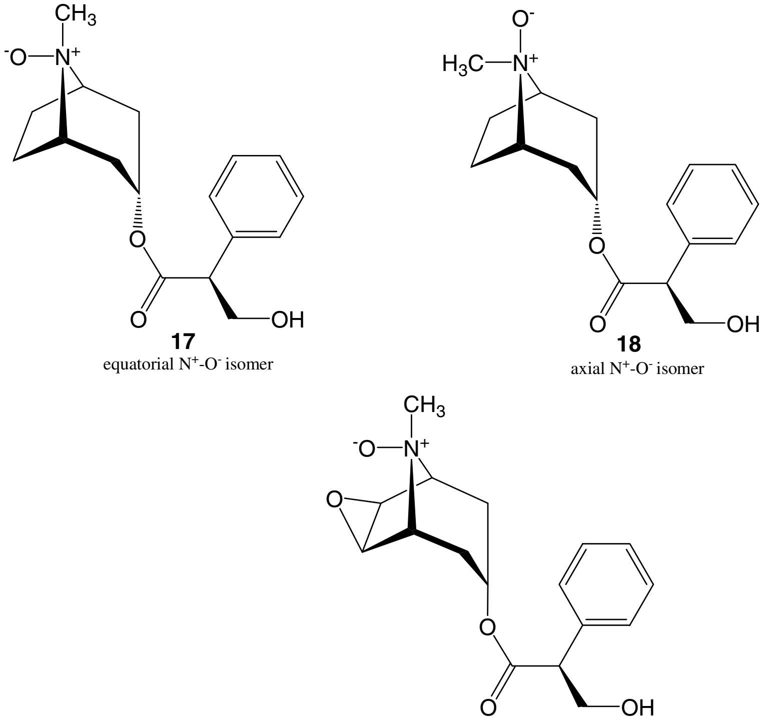


Fig. 2. Esters of tropane alkaloids.

could not be detected in the fresh roots. For the first time two tigloyl esters of hydroxytropane, 3α-tigloyloxytropane (**15**) and 3,6-ditigloyloxytropane (**16**) were identified.

Phillipson and Handa (1975) separated and characterized the isomeric *N*-oxides of (−)-hyoscyamine (**17**, **18**) and hyoscine (**19**). Two *N*-oxides (**17**, **18**) have been isolated from species of *Atropa*, *Datura*, *Hyoscyamus*, *Scopolia*, and from the roots, stems with leaves and fruits *Mandragora officinarum* L. (synonymous *M. officinalis*). The *N*-oxide (**19**) has been isolated only from species of the first four genera (see Fig. 3).

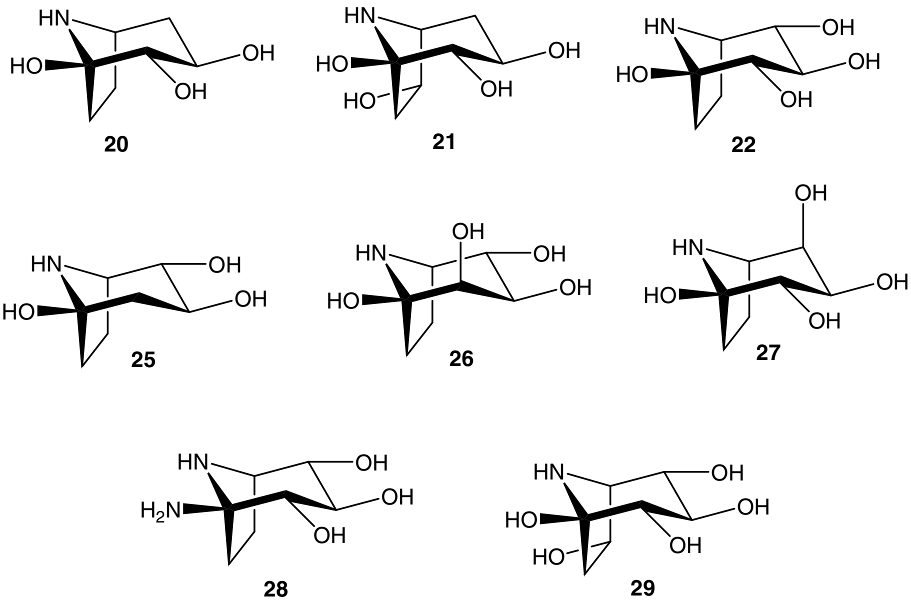


17
equatorial N^+-O^- isomer

18
axial N^+-O^- isomer

19

Fig. 3. *N*-Oxides of tropane alkaloids.



20 **21** **22**

25 **26** **27**

28 **29**

Fig. 4. Calystegines.

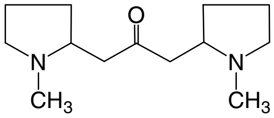


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Fig. 5. Cuscohygrine.

The chemistry of the European species of mandragora was fully described in a book chapter by Jackson and Berry (1979). Bekkouche et al. (1994) investigated by capillary gas chromatography atropine (**1**) and scopolamine (**7**) in different parts of *M. autumnalis* from Morocco. The highest atropine (**1**) level was found in the roots of samples during the flowering period of the plant.

Dräger et al. (1995) examined *M. officinarum* extracts (roots, leaves, spring leaves, flowers and fruits) for calystegine A3 (**20**) and calystegines B1 (**21**) and B2 (**22**). They found in aerial parts of the plant more calystegines than in the root. The highest content was in spring leaves and the lowest in fruits (see Fig. 4).

Al-Khalil and Alkofahi (1996) identified from the roots, leaves, unripe fruits and seeds of *M. autumnalis*, which grows naturally in Jordan, seven alkaloids, atropine (**1**), scopolamine (**7**), scopine (**8**), hyoscyamine (**9**), cuscohygrine (**11**), apoatropine (**12**), and belladonnines (**13**, **14**) (see Fig. 5).

Razzakov et al. (1998) studied leaves, whole surface parts, roots, rhizomes, fruits in different periods of vegetation, and seeds of *M. turcomanica* Mizger. They isolated scopolamine (**7**), hyoscyamine (**9**), and apohyoscine (**23**). After isolation of the main alkaloid, TLC and MS proved norhyoscyamine (**10**), apoatropine (**12**), α- or β-belladonnine (**13**, **14**), (±)-3α-tigloyloxytropan-6β-ol (**15**), tigloidine (3β-tigloyloxytropane) (**24**), and three another unidentified alkaloids (with molecular weight 209, 219 and 333).

Bekkouche et al. (2001) investigated by gas chromatography coupled to mass spectrometry the distribution of eight calystegines (A3 (**20**), A5 (**25**), B1 (**21**), B2 (**22**), B3 (**26**), B4 (**27**), C1 (**28**), and N1 (**29**)) in several different plants. They proved in *M. autumnalis* the most frequently encountered calystegines, A3, B1, B2, and B3. In the leaves they found calystegine B2 and B3 and in the roots A3, B1, B2, and B3.

3. Volatile and odor compounds

The odoriferous constituents of the fruits of *M. officinarum* were studied for the first time by Fleisher and Fleisher (1992, 1994). The volatile constituents of fully ripe fruits were analyzed by capillary GC–MS and 55 components were observed. Ethyl butyrate (22%), hexanol (9%), and hexyl acetate (7%) were found to be major among them. In addition, an unusually high proportion (over 7%) of sulfur compounds, which contributes to the heavy, fruity, and provocative odor of the mandrake fruit, was also characterized. Even-numbered γ-lactones were also found to make considerable contributions to the aroma.

Baser et al. (1998) analyzed by the headspace GC–MS samples of fresh ripe fruits of *M. autumnalis*. More than 100 compounds were characterized in the essential oil with ethyl esters of middle chain acids as the major constituents. Unripe and ripe fruits of mandragora, collected

Table 1
Composition of major volatile compounds from *Mandragora autumnalis*

Compound	%
Acetic acid	0.64
Benzyl acetate	0.33
Benzyl benzoate	0.08
β-Ionone	0.13
Butyl butyrate	1.83
Butyl caprate	1.13
Butyl caprylate	2.22
Butyl cinnamate	0.54
Butyl laurate	0.72
Caprylyl acetate	0.95
Cinnamic acid	0.50
δ-Laurolactone (δ-dodecalactone)	0.13
Docosane	0.69
Eicosane	0.06
Ethyl caprate	6.15
Ethyl caproate	4.40
Ethyl caprylate	8.70
Ethyl cinnamate	0.60
Ethyl laurate	3.19
Ethyl methylpropanethioate	1.34
Ethyl myristate	0.30
Ethyl oleate	0.53
Ethyl palmitate	0.26
Eugenol	1.03
Hexacosane	0.25
Hexadecane	0.60
Hexatriacontane	0.09
Hexyl 2-methylbutanoate	0.31
Hexyl caprylate	1.21
Isoeugenol	0.66
Isopropyl myristate	0.34
Lauric acid	1.62
Linoleic acid	6.56
Methyl caprate	0.53
Methyl laurate	0.26
Myristic acid	0.40
Neophytadiene	0.06
n-Hexyl acetate	6.50
n-Hexyl butyrate	3.02
Octacosane	0.40
Oleic acid	1.89
Palmitic acid	1.68
Pentadecane	0.11
Solavetivone	0.07
Squalene	0.64

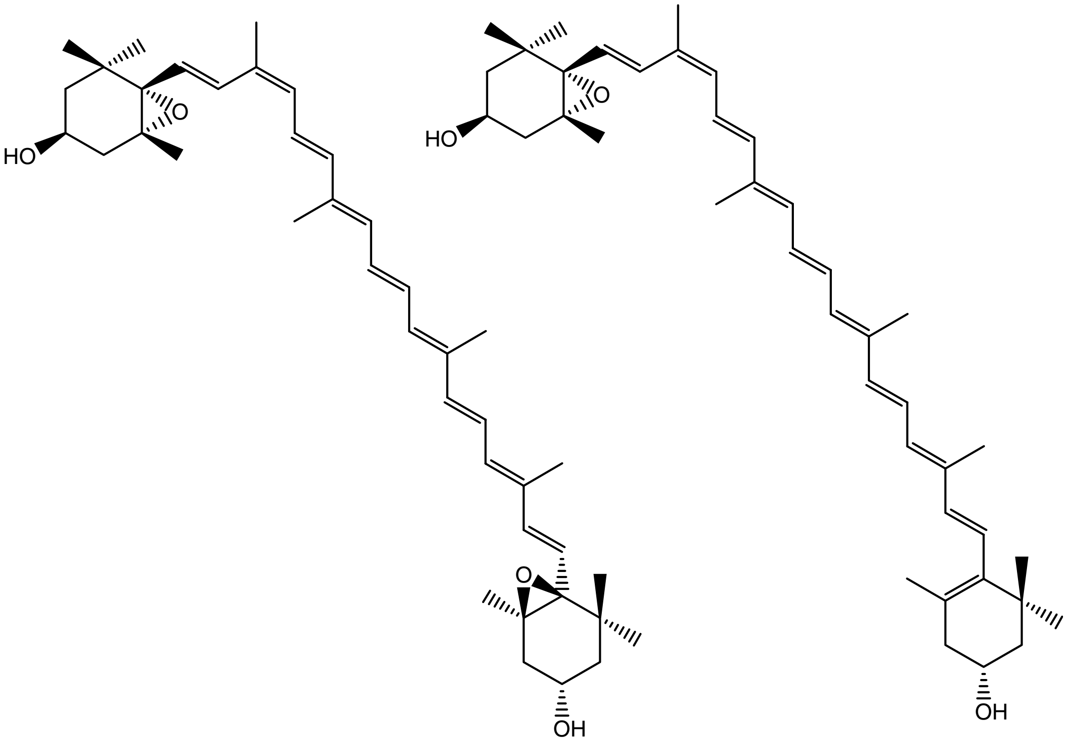
in the surroundings of Jerusalem, were analyzed. More than 130 compounds were identified by means of GC–MS; see Table 1 for the major components only.

4. Lipids and similar compounds

The four papers of Tashkent's group (Asilbekova et al., 1994; Gusakova and Asilbekova, 1997; Asilbekova and Glushenkova, 1998, 1999) describe the content of lipids and similar compounds from *M. turcomanica*. Lipids and lipid-like compounds were separated to 20 groups by column chromatography, preparative TLC, Ag^+-TLC, and were further identified by UV and IR spectroscopy, and mass spectrometry. Almost two-thirds of the total lipids were neutral lipids as hydrocarbons, complex esters of fatty acids with alkanols, triterpenols and sterols, triacylglycerols, oxoacyldiacylglycerols, free fatty acids, high molecular alcohols, hydroxyacyldiacylglycerols, 1,2- and 1,3-diacylglycerols, and monoacylglycerols. Polar lipids contained glycolipids (sterolglycosides, monogalactosyl- and digalactosyl-diacylglycerols) and phospholipids (phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and phosphatidic acid). Acylated sterols constituted esters of β-sitosterol, stigmasterol, campesterol, cholesterol, campesterol, lophenol, cycloartanol, cycloartenol, α-amyrin, β-amyrin, and 24-methylenecycloartanol. The authors found that the degree of saturation of the fatty acids in the triacylglycerols, phospholipids, and the free fatty acids of the fruit flesh was higher than those of the lipid classes of the seeds. The last paper (Asilbekova and Glushenkova, 1999) describes the structure of the triacylglycerols of the tuberous roots of *M. turcomanica.* Triacylglycerols include 42 species, among which the main ones are triunsaturated and unsymmetrical diunsaturated molecules with oleic acid in the glycerol molecule. The acylglycerols of the seeds flesh and roots differ not only in their fatty acid composition, but also in their position and type of triacylglycerol content. In the seeds are dominant molecules of triacylglycerols with linoleic acid in 2-, oleic and/or linoleic fatty acids in 1(3)-positions of glycerol and in the root analogous types with oleic and higher acids in *sn*-2-position.

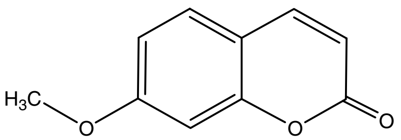
5. Pigments

Pakhlavouni et al. (2000) investigated carotenoid composition and chloroplast ultrastructure in two cell lines, M31-M and M31-P, of *M. turcomanica*. Despite high total chlorophyll and carotenoid contents in cells of both lines, grana appeared only after the increase in the relative content of chlorophyll *b* and epoxy-carotenoids, violaxanthin (**30**) and antheraxanthin (**31**) (see Fig. 6).



30 **31**

Fig. 6. Carotenoids.



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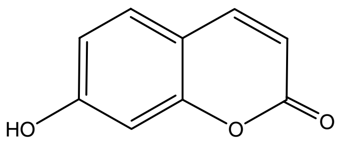

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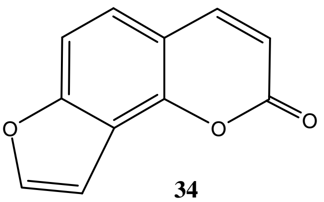

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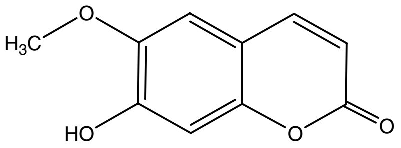

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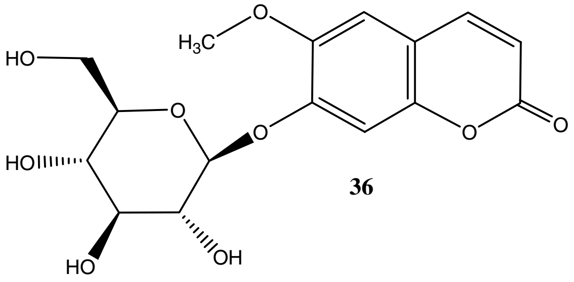

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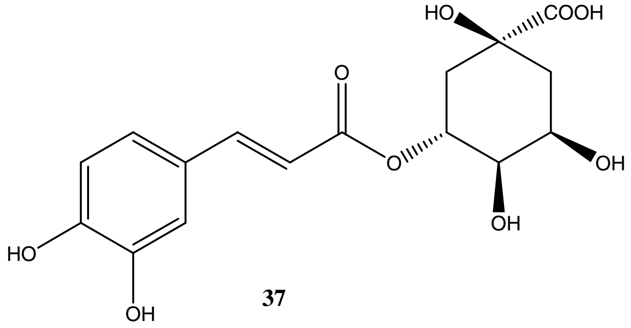

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Fig. 7. Coumarins and chlorogenic ester.

The ultrastructure of green plastids was more developed in M31-M callus than in the suspension culture of the same cell line. In the suspension culture of the M31-P cell line, granal thylakoids appeared in large (more than 700 μm) cell aggregates, pigment extracts from which contained antheraxanthin and violaxanthin. Chloroplasts exhibited symptoms of degradation: irregularly arranged lamellae, rare grana, numerous large osmiophilic globules, and electron-transparent vesicles in the matrix.

6. Coumarins

Al-Khalil and Alkofahi (1996) identified herniarin (**32**), umbelliferon (**33**), angelicin (**34**), scopoletin (**35**) and scopolin (**36**), and chlorogenic acid (**37**) from the roots, leaves, unripe fruits, and seeds of *M. autumnalis*, which grows wildly in Jordan valley. In addition, Staub (1942) investigated *M. autumnalis* roots for non-alkaloidal constituents and identified tropic acid (**3**) and scopoletin (**35**).

7. Conclusion

The presented review gives us the present state of knowledge in the chemistry of all different parts of mandragora plant. As we can see, the literature in this area is not abundant, but its contribution is significant. The chemical principle of its “magic” (hallucinogenic and poisonous) properties is today well known, but to understand its “aphrodisiac” (“love”) properties, still some more analytical work would be done (see Fig. 7). Further research can bring new and unexpected information.

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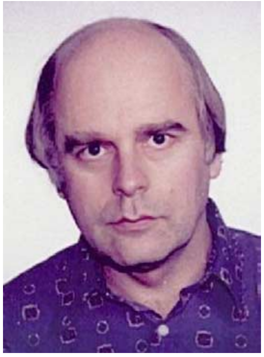

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